A MODIFIED AND IMPROVED METHOD FOR ISOLATION OF METAGENOMIC DNA FROM HILLY AND DENSE FOREST SOIL SAMPLES

Reena Singh[1], Vishnu Gupta, Vijeshwar Verma[2] and Shafaq Rasool [3*]

[1,2,3] *School of Biotechnology, Shri Mata Vaishno Devi University, Katra, 182320*
Jammu and Kashmir, (India)

ABSTRACT

A modified and efficient method has been developed for extracting high molecular weight (HMW) DNA free of inhibitors (humic substances) from hilly and dense forest soil samples. The method exhibited good yield and significantly improved the quality of DNA with efficient removal of humic substances. DNA extracted from different samples could be purified in one hour with 2% $CaCl_2$ treatment resulting in DNA pure enough to be used for gene cloning techniques. This paper presents in detail the method which can be used in overcoming the limitation of most of the reported protocols for soil DNA extraction and purification

Keywords -- *$CaCl_2$, HMW, Humic substances, Metagenome, PCR, PEG 8000*

I.INTRODUCTION

Microbes are the most ubiquitous organisms on earth, represented in all habitats and have a long history as resources for novel enzymes, biocatalysts and biologically active compounds [1].The concept of microbial diversity has dramatically expanded within the past decade. According to Amann [2] and colleagues only a minority of the micro-organisms living in any given habitat are cultivable. Amann reported that 0.001%-0.1% of the micro-organisms in
seawater, 0.25% in freshwater, 0.25% in sediments and only 0.3% of soil micro-organisms were found to be cultivable [1, 3]. Soil is the most diverse of all the naturally occurring microbial habitats with thousands of different microbial species in a few grams. There is a great opportunity for discovering new groups of micro-organisms that would be important for industrial and pharmaceutical research. It is not possible to recreate all of the specific requirements that all soil micro-organisms need, that is why, in addition to standard microbiological techniques, innovative molecular genetics methodologies are being designed and employed [4, 5,6,7].By applying these techniques to a given environment, one can obtain large quantities of genetic material and study a vast part of a given microbial community. Hence, soil is, and will continue to be an important source of useful micro-organisms awaiting discovery. Extraction of DNA from soil has enabled the biotechnologists to circumvent the need of culturing indigenous microbiota for metagenomic library construction. In metagenomic analysis the initial step is the isolation of intact, highly pure and high molecular weight (HMW) DNA [8].However, co-extraction of humic acids and other phenolic compounds is a major problem as they interfere

with downstream processing [9].Numerous methods have been recommended to remove humic substances from soil DNA including Cesium chloride density centrifugation [10], Cetyl-trimethyl-ammonium bromide (CTAB)[11], Polyvinylpolypyrrolidone (PVPP) [9], gel electrophoresis [9] and the Sephadex G- 200 column [12]. However, most of these methods are expensive and some result in reduced DNA recovery [13,15].

In the present study we describe (i) a modified method for soil DNA extraction and (ii) recommend a new method for removal of humic substances. The objectives of the study were to compare the efficiency of our method with other widely used DNA extraction and purification protocols.

II. MATERIALS AND METHODS

2.1 Collection of Soil Samples

Different environmental samples, encoded S1 and S2 were collected from Patnitop and Nathatop (Longitude 74.85 90''N, Latitude 32.731''E) at an altitude of 1500M and 2740M, and BGS3, SMVDUS4 from Bhairov Ghati and Shri Mata Vaishno Devi University (Longitude 32º 56 29''N, Latitude 74º 57 14''E) at an altitude of 1700M respectively of Jammu and Kashmir region in North Western Himalayas, India. The sample collection sites are exposed to wide range of temperatures in different seasons varying between 8 to 30ºC in summer & -10 to 10º C in winter. Approximately 1kg of soil was taken from the top (20cm) using a sterilized and autoclaved stainless steel knife. Samples were placed in sterilized polyethylene bags, placed in ice for transport and stored at 4ºC for further analysis.

2.2 Characteristics of the Soil

Soil moisture contents were determined by drying at 103ºC for 30 minutes and then calculating the percentage moisture content of the soil [14]. Soil pH was determined by diluting the soil sample up to (10^{-5}× dilution) in water and the pH of the sample was determined using pH metre.

2.3 Optimized Protocol for Soil DNA Extraction and Purification

5gm of soil was mixed with 13.5 mL of DNA extraction buffer [100 mM Tris-HCl (pH 8.0), 100 mM EDTA (pH8.0), 100 mM sodium phosphate (pH 8.0), 1.5 M NaCl, 1% CTAB] and 100µL of proteinase K (10 mg/mL)} into a sterile 50mL centrifuge tube. The tubes were incubated at 37ºC for 30 minutes with horizontal shaking. 1.5mL of 20% SDS (w/v) was added, and the samples were incubated at 65ºC for two hours with gentle inversions every 20 minutes. The tubes were centrifuged at 6,000*g* for 10 minutes at room temperature and the supernatant was transferred to fresh 50mL centrifuge tubes. The DNA was further extracted (II & III extractions) by adding 4.5 mL of extraction buffer and 0.5 mL of 20% SDS (w/v) followed by incubation at 65ºC for 15 min.The tubes were centrifuged at 6,000g for 10 min.The supernatants were mixed with an equal volume of Chloroform: Isoamyl alcohol (24:1).The aqueous phase was recovered by centrifugation at 15,000g for 10 minutes. The crude DNA was precipitated with 0.5 volumes of 50% PEG and 0.1 volume of 1M NaCl at -20ºC for one hour. The tubes were centrifuged at maximum speed and the DNA pellet was washed with 70% ethanol, dried and dissolved in TE (pH 8.0).

2.4 Removal of Humic Acid

DNA extracted by the optimized method was treated with 2% $CaCl_2$ and left at room temperature for one hour.

The samples were pelleted by centrifugation at 15,000g for 15 minutes to remove the co- extracted humic compounds. The supernant was collected and the DNA was precipitated with 1/10 volume of 7.5M ammonium acetate and two volumes of ethanol.The DNA was harvested at 16,000g for 20 minutes at room temperature. The pellet was air dried and resuspended in sterile 1X TE buffer (pH 8.0). The integrity of the DNA was checked on 0.8% (w/v) agarose gel. The estimations obtained from the gel were then correlated to the readings made by spectrophotometric analysis (UV 3000^{+}, Labindia Analytical Instruments Pvt. Ltd. New Delhi, India). Alternatively, DNA was also extracted by using different commercially available kits (Ultraclean ^{TM} [Mo Bio Laboratories Inc., Carlsbad, CA, USA], and Hiper^{TM} Soil DNA kit[Himedia Laboratories Pvt Ltd., Mumbai, India]) and various recommended protocols with slight modifications in our laboratory, a summary of which is shown in Table 1 along with references.

2.5 DNA quantification

After the purification, DNA was quantified by spectrophotometry with a UV/VIS spectrophotometer (Labindia Analytical Instruments Pvt. Ltd. New Delhi, India). The spectrophotometer was calibrated with lambda DNA,non methylated (Bangalore Genei, India).DNA concentration was determined at a wavelength of 260nm.The absorbance ratio A_{260}/A_{230} (DNA/humic acids) and A_{260}/A_{280} nm (DNA/protein) was used to evaluate the purity of extracted soil DNA [19].Considering the persistent co-extraction of humic acids from environmental samples we chose 1.5 as the minimum A_{260}/A_{230} nm ratio for a sample to be called, significantly purified.

2.6 PCR amplification

To further validate the purity of extracted soil DNA by the optimized protocol, PCR amplification was conducted for all the samples using16S rDNA primers 16Sf [5'-GAATT TGATCCTGGCTCAG-3'] and 16Sr [5'-GGCTACCTTGTTACGACTT-3'] [16] using Archael primers Arch-1F: 5'-TTC CGG TTG ATC CYG CCG GA-3' and Arch-1R: 5'-YCC GGC GTT GAM TCC AAT T- 3',including a control. PCR was performed in a total reaction volume of 20µL containing 1X Taq buffer (Bangalore Genei, India), 0.2mM of each dNTP, template DNA (1:10) diluted, and 1.5U Taq DNA polymerase (Bangalore Genei, India). PCR amplification was performed in a thermo cycler (Bio-Rad, Hercules, CA, USA) The optimized PCR condition was: Initial denaturation at 94ºC for 3 mins, 30 cycles of 94ºC for 30s, 55ºC for 1 min, 72ºC for 2 min and a final extension of 72ºC for 5 mins.The PCR products were subjected to electrophoresis on a 1% agarose gel containing 1kb DNA ladder (Fermentas Life Sciences,Carlsbad,CA,USA)

2.7 ITS Amplification

PCR amplification using ITS primers was performed with the environmental samples in order to determine the community of microbes present in the samples. The optimized PCR condition was: Initial denaturation at 94ºC for 2 mins, 30 cycles of 94ºC for 30s, 50ºC for 1 min, 72ºC for 1 min 30 seconds and a final extension of 72ºC for 10 mins.The PCR products were subjected to electrophoresis in a 1% agarose gel containing 1kb DNA ladder (Fermentas Life Sciences,Carlsbad,CA,USA)

2.8 Restriction enzyme digestion

Partial restriction digestion of the purified soil DNA samples was performed. DNA was digested with 0.5U of *Sau*3AI (Merck Specialities Pvt.Ltd., Mumbai, India) in an appropriate buffered 20ul reaction for 15, 20, 30, 45 and 1 hour at 37ºC.The enzyme was then heat inactivated at 65ºC for 5 min and the digested DNA was resolved in a 1% agarose gel with *Eco*RI and *Hind* III digested lambda DNA ladder (Fermentas Life Sciences, Carlsbad, CA, USA).

2.9 Construction of Metagenomic Library

2-6 kb fragments of partially digested metagenomic DNA were ligated into *Bam*H1 digested and dephosphorylated pUC $_{18}$ vector.The ligated products were then transformed into chemically competent *E.coli* DH5α cells. The transformation mixture was plated onto LB ampicillin plates containing X-gal (40ug/ml) and IPTG (50ug/ml) and the recombinants (white colonies) obtained were grown on LB plates containing 1.5% starch and ampicillin (100ug/ml).

III. RESULTS

The physico-chemical characteristics of the different soil samples collected from Patnitop, Nathatop, Bhairov Ghati and Shri Mata Vaishno Devi University regions of Jammu and Kashmir used in DNA extraction study have been listed in (Table 1).Soils were classified as sandy loam, loam, clay and loamy clay. The percentage of the moisture content was determined between 32.67% and 44.4%. The pH of the soil ranged from 7.1 to 7.7. The quality and yield of DNA extracted from 5g of soil samples by different extraction and purification procedures was compared with the optimized protocol (Table 1). Pretreatment with calcium chloride and aluminium ammonium sulphate failed to remove humic substances. Pre-treatment with aluminium ammonium sulphate and ammonium sulphate was not suitable for efficient humic acids removal, however the post treatment with aluminium sulphate enabled partial removal of humic substances, but the DNA yield was low. DNA extracted by CTAB extraction buffer method lead to insignificant removal of humic acids along with persistent degradation of DNA. DNA extracted by CTAB extraction buffer method [9] with 10% polyvinylpolypyrrolidone treatment resulted in no removal of humic content of the samples. In yet another modification of DNA extraction by Zhou et al; protocol DNA extracted was incubated with 2% $CaCl_2$ overnight however, partial removal of humic acid was seen The highest yield and the most efficient removal of humic acids was observed using CTAB extraction buffer method coupled with PEG/ NaCl precipitation and $CaCl_2$ purification (Fig 1). To evaluate DNA purity for enzyme digestion and PCR amplification spectrophotometric analysis was performed, and, A_{260}/A_{230} and A_{260}/A_{280} nm ratios were determined (Table 2). The results indicated that the modified method yielded DNA free of inhibitory humic compounds. In addition, the extraction of soil DNA by other methods and commercially available kits (Table 1) resulted in low concentration of DNA and inefficient removal of humic acids from the soil samples.

Qualitative estimation for checking the purity of extracted DNA was analyzed by PCR as well as restriction digestion using restriction enzyme *Sau*3A1. The extracted DNA from different soil samples was amplified using 16S rDNA primers, Archael 16S rDNA primers and ITS primers (Fig 2a, 2b and 2c) to represent the community

of microbes in these different samples. The metagenomic DNA isolated from different soil samples was digested with restriction endonucleases without giving any problems (Fig 3 and 4). The aim was to isolate the pure DNA from the soil samples which could be used for the construction of metagenomic libraries in *E.coli*.A library of approximately 15,000 colonies was constructed in *E.coli* using the isolated DNA in pUC_{18} vector.The library was screened for amylase activity using functional screening assay. Two clones were found positive among the 15,000 colonies screened so far (Figure 5). The clones are being further characterized in order to exploit their biotechnology potential.

IV. DISCUSSION

Theoretically, the microbial DNA, isolated from a soil sample represents the collective DNA of all the indigenous soil microorganisms [17,18]. In a broad range the DNA strategies are separation of cells and direct lysis [7].The main problem when isolating DNA from soil is co-precipitation of contaminating substances (humic compounds & phenolics) that interfere with downstream processes [19]. Although there are many methods published worldwide for the extraction of soil DNA and many commercial kits are available as well, the problem mentioned above is usually encountered in these protocols as physico-chemical properties of soil vary from location to location.

One of the most accepted methods i.e., CTAB extraction buffer method was followed [9]. The method used the detergents like CTAB and SDS for the direct cell lysis. This method could extract the DNA somewhat efficiently but could not remove the humic acid content. The high content of humic acid could interfere with other processes like PCR amplification and restriction digestion. To remove the humic acids different pre and post treatments with aluminium sulphate, aluminium ammonium sulphate, calcium carbonate and PVPP were given in conjunction with CTAB extraction buffer but humic acid was not efficiently removed..One more method of mechanical lysis by bead beating [8] was also used but the method showed negligible removal of humic acids . Most promising method seemed to be extraction by modified CTAB extraction buffer method complemented with 0.5 volumes of 50% PEG and 0.1 vol of 1M NaCl precipitation and purification by one step post treatment with 2% $CaCl_2$ .The role of $Cacl_2$ in purification of the extracted soil DNA is that it prevents the humic substances, to undergo oxidation forming quinones, which covalently bind to the DNA, thus hampering the DNA and Taq polymerase interaction. The gel analysis as well as spectrophotometric analysis showed high concentration of DNA and efficient removal of humic substances .

Inhibition of PCR is often sourced to DNA contaminated with humic acids co-extracted from the soil [3, 20, 21]. The efficacy of the method described in this paper to remove humic acids was verified by 16S rDNA PCR amplification and to demonstrate that the isolated soil DNA represents community of micro-organisms PCR amplification was performed with ITS primers The purified DNA samples were then successfully partially digested using restriction endonuclease *Sau*3A1. The restriction digestion was optimised in order to yield 2-6kb fragments. The protocol optimized in the laboratory worked efficiently on all the tested soil types and metagenomic library of approximately 15,000 clones in *E.coli* was constructed. Some of the clones were found positive for amylase activity.

V. CONCLUSION

Hence, the soil DNA extraction and purification method described here, is a simple and efficient method for most of the soil samples. DNA extracted from different samples could be purified in one hour with 2% $CaCl_2$ treatment, resulting in DNA pure enough to be used for restriction digestion and PCR, as compared to the commercially available kits in the market and other reported methods which resulted in lower concentration of DNA along with only 60-70% success rate in PCR amplification of the extracted soil DNA. Thus, the optimized laboratory method yielded higher concentration of DNA free of humic substances enabling us to construct metagenomic library meant for selecting clones that exhibit amyloytic activity.

VI. ACKNOWLEDGEMENT

The work was financially supported by Council of Scientific and Industrial Research (CSIR), New Delhi, Government of India; Project No. 37(1545)/12/EMR-II entitled "Exploring Microbial Diversity and Mining Novel Hydrolases from Brackish Water Lakes of Ladakh Region by Metagenomic Approach". *Miss Reena Singh is thankful to Council of Scientific and Industrial Research (CSIR), Government of India for the award of Senior Research Fellowship.

VII. REFERENCES

[1] P. Lorenz , and J. Eck , Metagenomics and industrial applications, *Nat Rev Mic robiol, 3, 2005, 510–516.*

[2] R.I. Amann, W. Ludwig and K.H. Schleifer KH, Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiol Rev, 59, 1995, 143– 169.*

[3] P.R. Robe, C. Nalin, T.M. Capellano and V.P.Simonet, Extraction of DNA from soil. *Eur J Soil Biology, 39, 2003, 183-190.*

[4] I.G. Wilson, Inhibition and facilitation of nucleic acid amplification. *Appl Environ Microbiol, 6, 1997, 3741– 3751.*

[5] H.A. Iqbal, F. Zhiyang, and F.B. Sean, Biocatalysts and small molecule products from metagenomic studies. *Current Opinion in Chemical Biology, 16, 2012, 109-116.*

[6] M.I.More, J.B.Herrick, M.C.Silva, W.C. Ghiorse, and E.L.Madsen, Quantitative cell lysis of indigenous microorganisms and rapid extraction of microbial DNA from sediment. *Appl Environ Microbiol, 60, 1994, 1572– 1580.*

[7] C.L. Roose Amsaleg, S.E. Garnier, and M. Harry, Extraction and purification of microbial DNA from soil and sediment samples. *Appl Soil Ecol, 18, 2001, 47-60.*

[8] D.Dong, A.Yan , H.Liu, X. Zhang and Y.Xu, Removal of humic substances from soil DNA using aluminium sulphate. *J Microbiol Methods, 66, 2006, 217-222.*

[9] J. Zhou, M.A. Bruns, and J.M. Tiedie, DNA recovery from soils of diverse composition. *Appl Environ Microbiol, 62, 1996, 316-322.*

[10] L.G.Leff, J.R.Dana, J.V. Arthur and L.J. Shimkets, Comparison of methods of DNA extraction from stream sediments. *Appl Environ Microbiol, 61, 1995, 1141– 1143.*

[11] J. Cho, D. Lee, Y. Cho, J. Cho, and S. Kim, Direct extraction of DNA from soil for amplification of 16S rDNA gene sequences by polymerase chain reaction. *J Microbiol, 34, 1996, 229–235.*

[12] D.N.Miller, J.E. Bryant, E.L. Madsen and W.C. Ghiorse, (1999) Evaluation and optimization of DNA extraction and purification procedures for soil and sediment samples. *Appl Environ Microbiol, 65, 1999, 4715–4724.*

[13] M.I. More, J.B. Herrick, M.C. Silva, W.C. Ghiorse, and E.L. Madsen, Quantitative cell lysis of indigenous microorganisms and rapid extraction of microbial DNA from sediment. *Appl Environ Microbiol, 60, 1994, 1572– 1580.*

[14] A. Janessen, C. Sperl, W. Bouten , and J.M. Verstraten, Soil water content measurement at different scales: accuracy of time domain reflectometry and ground penetrating radar. *Journal of Hydrology, 245, 2001, 48-58.*

[15] C.R. Kusk, K.L. Banton, D.L. Adorada, P.C.Stark, and P.J. Jackson, Small-scale DNA sample preparation method for field PCR detection of microbial cells and spores in soil. *Appl Environ Microbiol 64, 1998, 2463–2472.*

[16] A. Matsuda, S. Tazumi, T. Kagawa, O. Sekizuka, J.E. Murayama, B.C.Moore, and Millar, Homogeneity of the 16S rDNA sequence among geographically disparate isolates of *Taylorella equigenitalis. BMC Veterinary Research 2, 2006, 1.*

[17] J. Handelsman, M.R. Rondon, S.F. Brady, J. Clardy, and R.M. Goodman, Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. *Chem Biol 5, 1998, R245–R249.*

[18] M.R. Rondon, R.M.Goodman, and J. Handelsman, The earth's bounty: accessing soil microbial diversity. *TIBTECH 17, 1999, 403–409.*

[19] Y.Tsai, and B.H. Olson, Rapid method for separation of bacterial DNA from humic substances in sediments for polymerase chain reaction. *Appl Environ Microbiol 58, 1992, 2292-5.*

[20] J.E.Stach, S. Bathe,J.P. Clapp and R.G. Burns, PCR-SSCP comparison of 16S rDNA sequence diversity in soil DNA obtained using different isolation and purification methods. *FEMS Microbiol ECOL 36, 2001, 139- 151.*

[21] C.C.Tebbe, and W. Vahjen , Interference of humic acids and DNA extracted directly from soil in detection and transformation of recombinant DNA from bacteria and a yeast. *Appl Environ Microbiol 59, 1993, 2657-2665.*

FIGURE LEGENDS:

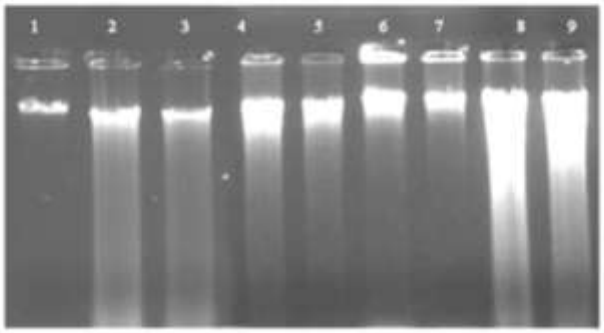

Fig 1: 0.8% Agarose gel electrophoresis of soil DNA extracted from different soil samples (PTS1, NTS1, BGS3 and SMVDUS4) by PEG and 2% $CaCl_2$ treatment. Lanes 1. Uncut λ DNA; 2. PTS1 PEG precipitated DNA; 3. PTS1 DNA treated with 2% $CaCl_2$; 4. NTS2 PEG precipitated DNA; 5. NTS2 DNA treated with 2% $CaCl_2$; 6. BGS3 PEG precipitated DNA; 7. BGS3 DNA treated with 2% $CaCl_2$; 8. SMVDUS4 PEG precipitated DNA; 9. SMVDUS4 DNA treated with 2% $CaCl_2$ (All 1st extraction).

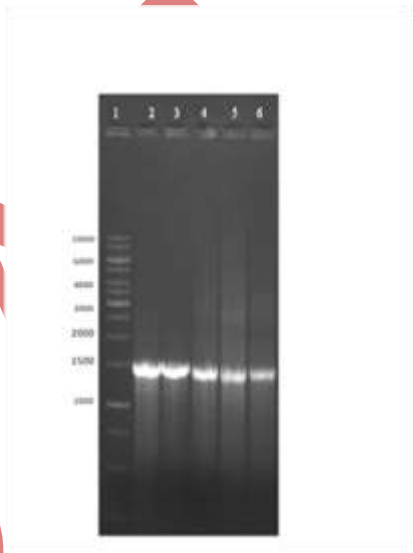

Fig 2(a): 1% Agarose gel showing 16S rDNA amplification products of extractions of PTS1, NTS2, BGS3 and SMVDUS4 DNA with PEG precipitation+ $CaCl_2$ treatment. Lanes. 1. 1kb DNA ladder; 2. Control; 3. PTS1 soil DNA; 4. NTS2 soil DNA; 5. BGS3 soil DNA; 6. SMVDUS4 soil DNA

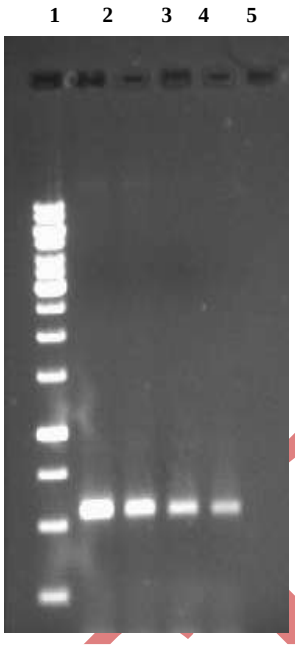

Fig 2(b): 1% Agarose gel showing amplification products of extractions of PTS1, NTS2, BGS3 and SMVDUS4 DNA with ITS primers. Lanes. 1. 1kb DNA ladder; 2. PTS1 soil DNA; 3. NTS2 soil DNA; 4. BGS3 soil DNA; 5. SMVDUS4 soil DNA

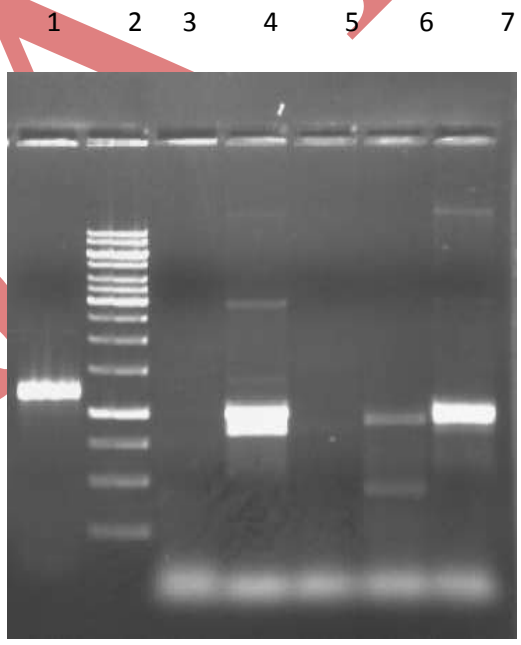

Fig 2(c): 1% Agarose gel showing PCR amplification of Metagenomic DNA of different soil samples using Archael primers 1.Positive control ,2. 1kb DNA ladder;3.Negative control ;4 PTS1 soil DNA; 5. SMVDUS4 soil DNA; 6. BGS3 soil DNA; 7. NTS2 soil DNA

Fig 3: 1% Agarose gel showing partial restriction digestion of PTS1 and NTS2 DNA. Lanes. 1. λ DNA cut with *Eco* RI/*Hind*III; 2. *Sau*3AI digested PTS1 DNA (45 mins); 3. *Sau*3AI digested NTS2 DNA (1Hr).

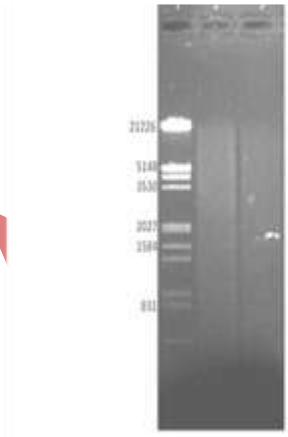

Fig 4: 1% Agarose gel showing partial restriction digestion of PTS1 and NTS2 DNA. Lanes. 1. λ DNA cut with *Eco* RI/*Hind*III; 2. *Sau*3AI digested BGS3 DNA (30 mins); 3. *Sau*3AI digested SMVDUS4 DNA (1Hr).

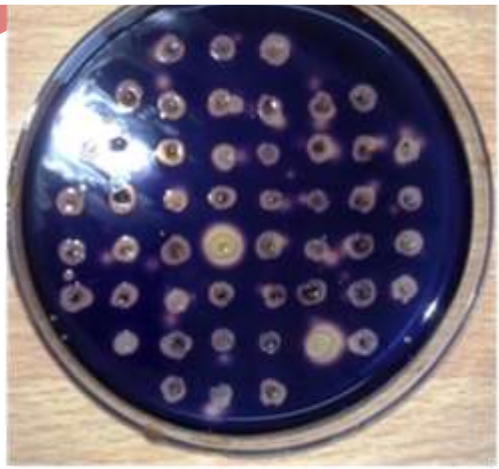

Fig 5: The two positive clones having clear zones of starch hydrolysis around the colonies indicates amylase secretion when flooded with iodine solution.

TABLE 1: Comparison of various soil DNA extraction and purification methods

S.No.	Method of DNA Isolation	Modifications done	Results	References
	1. Bead beating lysis	Pre- treatment with $CaCO_3$	Negligible amount of humic acid was removed. DNA obtained was sheared.	No modification S. Marketa. *et al;*2008
	2. By CTAB extraction buffer	Pre-treatment with aluminium ammonium sulphate	Humic acid was not removed	No modification S. Marketa. *et al;*2008
	3. By CTAB extraction buffer	Pre-treatment with aluminium sulphate	Low yield of DNA.	No modification Dong *et al;* 2006
	4. Extraction by using glass beads	Pre-treatment with Aluminium sulphate and Aluminium ammonium sulphate	Low yield of DNA. Humic acid was not removed	Modified Dong *et al;* 2006
	5. By CTAB extraction buffer	Post treatment with aluminium sulphate	Humic acid was partially removed and low yield of DNA.	Modified Dong *et al;* 2006
	6. By CTAB extraction buffer	Post treatment with $CaCO_3$	Negligible amount of humic acid was removed. DNA obtained was sheared	Zhou. *et. al*;1996 Modified S. Marketa. *et al;*2008

7. By CTAB extraction buffer	**Post treatment with 10% PVPP**	**Humic acid was not removed**	**Zhou. *et. al*;1996 Modified Krsek. *et. al*;1999 Nalin *et. al* 1999.**
8. By CTAB extraction buffer	**Post treatment with aluminium ammonium sulphate**	**Humic acid was not removed**	**No modification Zhou. *et. al*;1996 Braid.*et. al*; 2003**
9. By CTAB extraction buffer	**Overnight Post treatment with 2% $CaCl_2$.**	**Humic acid was not completely removed.**	**Modified Zhou. *et. al*;1996**
10. Mo Bio Kit, CA, USA	**----------**	**Low concentration of DNA**	**----------**
11. Hiper Soil DNA Kit, Himedia,India	**----------**	**Inefficient removal of humic acids**	**---------**
12. DNA extraction by using activated charcoal.	**Treatment with activated charcoal & modified extraction buffer**	**Low yield of DNA.**	**D.Verma. *et.al*;201 1**
13. DNA extraction by using glass beads	**Treatment with sodium phosphate buffer & sodium dodecyl sulphate.**	**Humic acid not removed**	**A.Ogram *et. al* ; 1987**
14. *By CTAB extraction buffer with 50% PEG and 1M NaCl (for soil samples)	**2% $CaCl_2$ post treatment with 1 hour incubation**	**Humic acid was efficiently removed and high yield of DNA.**	**This work**

***Work presented in this paper**

TABLE 2: Spectrophotometric analysis of extracted DNA from different soil samples

(A)

Soil Sample	$A_{260/280}$ ratio	$A_{260/230}$ ratio	Conc. (3gmL^{-1})
S1 (1)	1.720	1.663	2033
(2)	1.689	1.845	1140
(3)	1.740	1.750	1898

(B)

Soil Sample	$A_{260/280}$ ratio	$A_{260/230}$ ratio	Conc. (3gmL^{-1})
S2 (1)	1.761	1.634	4980
(2)	1.726	1.839	6060
(3)	1.801	1.799	1035

(C)

Soil Sample	$A_{260/280}$ ratio	$A_{260/230}$ ratio	Conc. (3gmL^{-1})
BGS3 (1)	1.698	1.602	5880
(2)	1.755	1.765	4750
(3)	1.652	1.832	2140

(D)

Soil Sample	$A_{260/280}$ ratio	$A_{260/230}$ ratio	Conc. (3gmL^{-1})
SMVDUS4 (1)	1.808	1.651	9270
(2)	1.712	1.874	5100
(3)	1.646	1.854	3060

Work presented in this paper