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Comparative Studies on the Extraction of Metagenomic DNA from Various Soil and Sediment Samples of Jammu and Kashmir Region in Prospect for Novel Biocatalysts

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Abstract: *A modified and efficient method has been developed for extracting high molecular weight (HMW) DNA free of inhibitors (humic substances) from soil and sediment samples of Jammu and Kashmir region. The method includes precipitation of extracted soil and sediment DNA with 0.5 volumes of 50% polyethylene glycol (PEG) and 0.1 volumes of 1M NaCl (sodium chloride) followed by treatment with 2% $CaCl_2$(calcium chloride).The results of this method were compared with the other reported procedures. The method exhibited good yield and significantly improved the quality of DNA with efficient removal of humic substances. The purity of the extracted DNA from soil and sediment samples was validated by restriction analysis and PCR amplification. DNA extracted from different samples could be purified in one hour with 2% $CaCl_2$ treatment resulting in DNA pure enough to be processesed for gene cloning techniques. Standard protocol of [9] was used for the extraction of DNA from brackish waters. This paper presents in detail the method which can be used successfully in overcoming the limitation of most of the reported protocols for soil and sediment DNA extraction and purification.*

Keywords: *Brackish, $CaCl_2$, 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] and [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].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. However, co-extraction of humic acids and other phenolic compounds is a major problem as they interfere with downstream processing [5].Numerous methods have been recommended to remove humic substances from soil DNA including Cesium chloride density centrifugation [6], Cetyl-trimethyl-ammonium bromide (CTAB),[7], Polyvinylpolypyrrolidone (PVPP), [8] and [9], gel electrophoresis [9] and the Sephadex G- 200 column [10]. However, most of these methods are expensive and some result in reduced DNA recovery [11] and [12].

70% of earth's surface is covered with water, which makes it sizeably the largest ecosystem on the planet. Water can provide the most extreme of habitats prevailing on earth. Its temperature can range from -70 ^{o}C in the Antarctic glaciers to over 100 ^{o}C in the natural hot springs of Bulgaria [13].Water bodies are known to harbour an enormous plethora of microbial population, which has previously been ignored for decades. Microbiologists as well as environmentalists worldwide have shown mushrooming interest in the micro biota of the aqueous ecosystem. This makes water a potential reservoir of various bio geochemically active principles, novel enzymes and genes essential for survival [7].

In the present study we describe (i) a modified method for soil and sediment 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

Collection of Soil, Sediment and Water Samples*:* Different soil, sediment and water samples were collected from different regions of Jammu and Kashmir, in North Western Himalayas, India coded as PTS1 and NTS2 from Patnitop and Nathatop respectively (Longitude 74.85 90''N, Latitude 32.731''E) at an altitude of 1500m and 2740m respectively, BGS3 and SMVDUS4 from Bhairov Ghati and Shri Mata Vaishno Devi University (Longitude 32^o 56 29''N, Latitude 74^o 57 14''E) at an altitude of 2650m and 754m respectively , SedTR lake sediment (Longitude 33^o 18'N, Latitude 77^o 59'E) at an altitude of 4530m and SedTM from Tsomoriri lake sediment (Longitude 32°54′N ,Latitude78°18′E 32.900°N) at an altitude of 4,595 m respectively.Water samples namely WTR, WTM, WTP were collected from Tsokar, Tsomoriri and Pangong brackish lakes (Longitude 33°43′04.59″N Latitude 78°53′48.48″E) at an altitude of 4,350 m of Ladakh region respectively. The sample collection sites are exposed to wide range of temperatures in different seasons varying between 4 to 30ºC in summer and -30 to 10º C in winter. Approximately 1kg of soil and sediment samples were taken from the top (20cm) using a sterilized and autoclaved stainless steel knife. Soil and sediment samples were placed in sterilized polyethylene bags and water samples were collected in nalgene screw capped (Thermo scientific; USA) sterilized bottles, placed in ice for transport and stored at 4ºC for further analysis.

Characteristics of the Soil, Sediment and Water*:* The moisture contents were determined by drying 1g soil and sediment at 103ºC for 30 minutes and then calculating the percentage moisture content of the soil and sediment .pH of soil and sediment was measured after diluting the samples in water, using pH meter. The pH of water samples was measured by pH meter.

Optimized Protocol for Soil DNA Extraction

5gm of soil and sediment 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).

Sediment DNA Extraction

Several methods were adopted for isolation of DNA from sediment samples (Table 2) and the results were compared with the optimized DNA extraction protocol.

Purification of Soil and Sediment DNA

Removal of Humic Acid: DNA extracted by the optimized method from soil and sediment 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 New Delhi; India).

Alternatively, DNA was also extracted by using different commercially available kits (Ultraclean ™ [Mo Bio Laboratories Inc., Carlsbad, CA, USA], and Hiper™ Soil DNA kit [Himedia Laboratories., Mumbai, India]) and various recommended protocols with slight modifications in our laboratory, a summary of which is shown in Table 2.

The soil and sediment samples were pre-treated with calcium carbonate and aluminium ammonium sulphate as reported by Sagova-Mareckova et al and the DNA was extracted by bead beating lysis [14].Dong and co-workers protocol of pre-treatment with aluminium sulphate was performed on the soil and sediment with various pH adjustments [15] and bead beating lysis was used to extract the DNA.

Modifying the method outlined by Dong et al, we pre-treated the soil and sediment samples with 50mM each of aluminium sulphate and aluminium ammonium sulphate and post treated the extracted DNA with 100mM aluminium sulphate. The method of Sagova-Mareckova et al; using calcium carbonate was modified in our laboratory.The DNA extracted by CTAB extraction buffer method [9] was treated with calcium carbonate.The DNA was precipitated with ethanol and dissolved in TE buffer pH (8.0). DNA extracted by CTAB extraction buffer method [9] was treated with 10% polyvinylpolypyrrolidone.DNA was precipitated with ethanol and dissolved in TE buffer pH (8.0). Another modification of DNA extraction protocol by Zhou et al; was made, where we incubated the DNA extracted from 5g of soil and sediment with 2% $CaCl_2$ overnight. The DNA was re- precipitated with ethanol and ammonium acetate and dissolved in TE buffer pH (8.0).

Water DNA Extraction and Purification

Approximately 2-5 litres of water collected from different brackish lakes was filtered through 0.45µm and 0.22µm PES membrane filter using a vacuum manifold and the DNA extraction was performed by following [9] protocol without any pre and post modifications. The extracted DNA from water samples was purified by using PCR purification kit (Qiagen; USA)

DNA quantification

After the purification, DNA was quantified by spectrophotometry with a UV/VIS spectrophotometer (Labindia Analytical Instruments New Delhi; India). The spectrophotometer was calibrated with lambda DNA,non methylated (Bangalore Genei; India).DNA concentration was determined at a wavelength of 260 nm.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, sediment and water DNA .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.

A modified and efficient method has been developed for extracting high molecular weight (HMW) DNA free of inhibitors (humic substances) from soil and sediment samples of Jammu and Kashmir region. The method includes precipitation of extracted soil and sediment DNA with 0.5 volumes of 50% polyethylene glycol (PEG) and 0.1 volumes of 1M NaCl (sodium chloride) followed by treatment with 2% $CaCl_2$(calcium chloride).The results of this method were compared with the other reported procedures. The method exhibited good yield and significantly improved the quality of DNA with efficient removal of humic substances. The purity of the extracted DNA from soil and sediment samples was validated by restriction analysis and PCR amplification. DNA extracted from different samples could be purified in one hour with 2% $CaCl_2$ treatment resulting in DNA pure enough to be processesed for gene cloning techniques. Standard protocol of [9] was used for the extraction of DNA from brackish waters. This paper presents in detail the method which can be used successfully in overcoming the limitation of most of the reported protocols for soil and sediment DNA extraction and purification.

To further validate the purity of extracted soil and sediment DNA by the optimized protocol, and water DNA by [9] procedure, PCR amplification was conducted for all the samples, 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, 16S rDNA primers 16Sf [5'-GAATT TGATCCTGGCTCAG-3'] and 16Sr [5'-GGCTACCTTGT TACGACTT-3'] [16] 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 conditions were: 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 in a 1% agarose gel containing 1kb DNA ladder (Fermentas Life Sciences, Carlsbad, CA; USA)

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 Mumbai; India) in an appropriate buffered 20µl 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).

Construction of Metagenomic Library

2-6 kb fragments of partially digested soil metagenomic DNA were ligated into *Bam*H1 digested and dephosphorylated pUC $_{18}$ vector.The ligated products were then transformed into chemically competent *E.coli* Top 10 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, sediment and water samples collected from Patnitop, Nathatop, Bhairov Ghati, Shri Mata Vaishno Devi University, Tsokar lake sediment, Tsomoriri lake sediment, Tsokar, Tsomoriri and Pangong brackish lakes of Ladakh region respectively, 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 of the soil and sediment was determined between 32.67% and 45.2%. The pH of the soil and sediment ranged from 7.1 to 9.0. The pH values of the water samples were in alkaline range (8.8-9.5).

The quality and yield of DNA extracted from 5g of soil and sediment samples by different extraction and purification procedures was compared with the optimized protocol (Table 2). 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 of soil samples 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 of soil and sediment samples 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 and Fig 2).An increase in yield and purity of DNA isolated from water samples was observed using Zhou et al protocol. 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 3). The results indicated that the modified method yielded DNA free of inhibitory humic compounds. In addition, the extraction of soil and sediment DNA by other methods and commercially available kits (Table 2) resulted in low concentration of DNA and inefficient removal of humic acids from the soil and sediment 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 was amplified by using 16S primers (Fig 4) and the soil DNA was digested without giving any problems (Fig 5a and 5b).

Different soil characteristics correspond to changes in PCR amplification [14]. Sharp amplification of 16Sr DNA fragments (approx. 1.5kb) was observed when purified DNA samples were used as template. (Fig 4a).

Our aim was to isolate the pure DNA from the soil and sediment 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 (Fig. 6). The clones are being further characterized in order to exploit their biotechnology potential.

IV. Discussion

Theoretically, the microbial DNA, isolated from a soil, sediment and water sample represents the collective DNA of all the indigenous environmental microorganisms [17] and [18]. In a broad range the DNA strategies are separation of cells and direct lysis [19].The main problem when isolating DNA from soil and sediment is co-precipitation of contaminating substances (humic compounds & phenolics) that interfere with downstream processes[20]. Although there are many methods published worldwide for the extraction of soil and sediment 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 and sediment vary from location to location.

We started with one of the most accepted methods i.e., CTAB extraction buffer method [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. (Table 2). 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. (Table 2). One more method of mechanical lysis by bead beating [5] was also used but the method showed negligible removal of humic acids (Table 2). 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% $CaCl_2$ (Table 2).The role of $CaCl_2$ in purification of the extracted soil and sediment 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 (Fig1, 2 and Table 3). Gel analysis of extracted water DNA confirmed Zhou et al 1996 method to be the most suitable protocol for isolation of water DNA from brackish lakes (Fig 3). Inhibition of PCR is often sourced to DNA contaminated with humic acids co-extracted from the soil [18], [23] and [25]. The efficacy of the method described in this paper to remove humic acids was verified by 16S rDNA PCR amplification. 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 protocols optimized in our laboratory worked efficiently on all tested soil, sediment and water types and we were able to construct the soil metagenomic library of approximately 15,000 clones in *E.coli.* Some of the clones were found positive for amylase activity. (Fig 6).

V. Figures And Tables

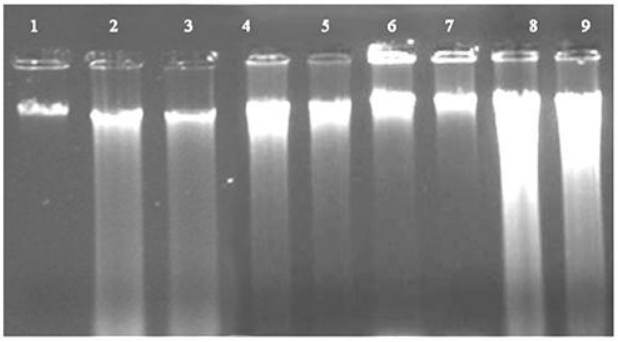

Fig 1: 0.8% Agarose gel electrophoresis of soil DNA extracted from different soil samples (PTS1, NTS1, BGS3 and SMVDUS4) by PEG and 2% $CaCl_2$ treatment. Lanes. 1. Uncut λ DNA; 2. PTS1 PEG precipitated DNA; 3. PTS1 DNA treated with 2% $CaCl_2$;4. NTS2 PEG precipitated DNA; 5. NTS2 DNA treated with 2%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

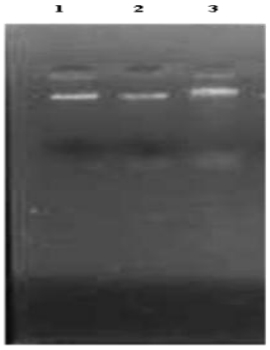

Fig 2: 0.8% Agarose gel electrophoresis of sediment DNA extracted from different sediment samples (SedTR and SedTM) by2% $CaCl_2$ treatment. Lanes. 1. Uncut λ DNA; 2. SedTR DNA treated with PEG + 2% $CaCl_2$; 3. SedTM DNA treated with PEG + 2% $CaCl_2$

Fig 3: 0.8% Agarose gel electrophoresis of water DNA extracted from different Brackish lakes (TWP, TWM and TWR). Lanes. 1. TWP extracted DNA; 2. TWM extracted DNA; 3. TWR extracted DNA

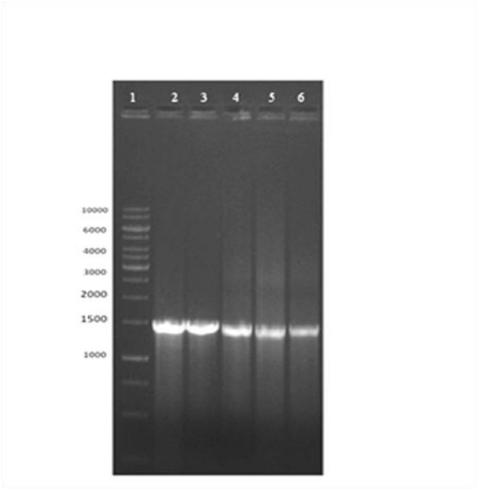

Fig 4: 1% Agarose gel showing 16S rDNA amplication products of extractions of PTS1, NTS2, BGS3 and SMVDUS4 DNA with PEG precipitation + $CaCl_2$ treatment. Lanes. 1. 1kb DNA ladder; 2. Control; 3.PTS1 soil DNA; 4. NTS2 soil DNA; 5.BGS3 soil DNA; 6. SMVDUS4 soil DNA

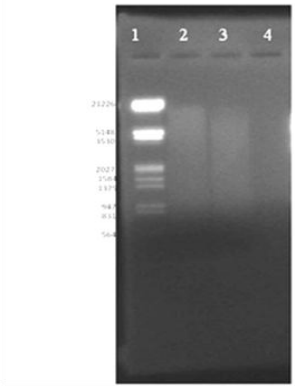

Fig 5(a): 1% Agarose gel showing partial restriction digestion of PTS1 and NTS2 DNA. Lanes. 1. λ DNA cut with *Eco*R I/*Hind*III. 2. *Sau*3AI digested PTS1 DNA (45 mins); 3. *Sau*3AI digested NTS2 DNA (1Hr).

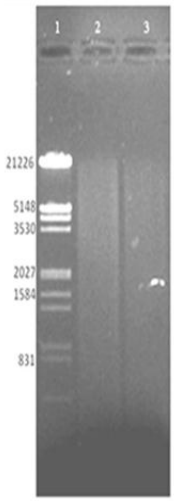

Fig 5(b): 1% Agarose gel showing partial restriction digestion of BGS3 and SMVDUS4 DNA. Lanes. 1. λ DNA cut with *Eco*R I/*Hind*III. 2. *Sau*3AI digested BGS3 DNA (30 mins); 3. *Sau*3AI digested SMVDUS4 DNA (1Hr).

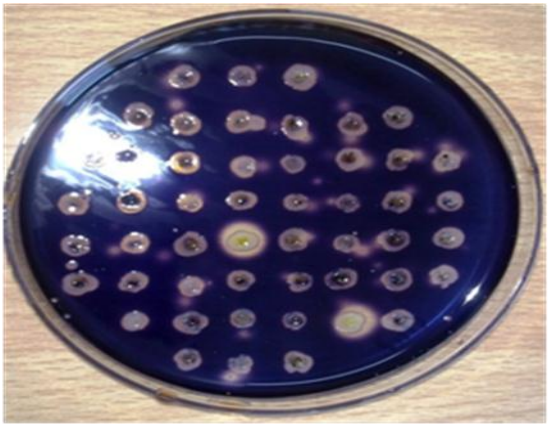

Fig 6: The two positive clones having clear zones of starch hydrolysis around the colonies indicates amylase secretion when flooded with iodine solution.

TABLE 1: Physico-chemical analysis of different soil, sediment and water samples collected from Patnitop, Nathatop, Bhairov Ghati, Shri Mata Vaishno Devi University, Tsokar lake sediment Tsomoriri lake sediment and water samples from Tsokar, Tsomoriri and Pangong brackish lakes respectively of Ladakh region of the state of Jammu & Kashmir

S.No.	Samples	Texture	Moisture content	pH
1.	PTS1	Sandy loam	35.94%	7.4
2.	NTS2	Loam	44.4%	7.7
3.	BGS3	Clay	43.0%	7.0
4.	SMVDUS4	Loamy clay	32.67%	7.1
5.	SedTR	-	44.4%	8.8

6.	SedTM	-	45.2%	9.0
7.	WTR	Turbid	100%	8.8
8.	WTM	Clear	100%	9.0
9.	WTP	Clear	100%	9.4

PTS1: Patnitop Soil, NTS2: Nathatop Soil, BGS3: Bhairov Ghati Soil, SMVDUS4: Shri Mata Vaishno Devi University Soil, SedTR: Tsokar Sediment lake Soil, SedTM: Tsomorriri Sediment Lake Soil, WTR: Tsokar Water, WTM: Tsomorriri Water, WTP: Pangong Water.

TABLE 2: Comparison of various soil and sediment 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 extraction buffer	Pre-treatment with aluminium ammonium sulphate	Humic acid was not removed	No modification S. Marketa. et al;2008
3.	By 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 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 Inefficient removal of humic acids	---
11.	Hiper Soil DNA Kit,Himedia,India		Low concentration of DNA 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;2011
13.	DNA extraction by using glass beads.	Treatment with sodium phosphate buffer & sodium	Humic acid not removed	A.Ogram et. al ; 1987

		dodecyl sulphate.		
		2% $CaCl_2$ post treatment with 1 hour incubation	Humic acid was efficiently removed and high yield of DNA.	
14*	*By CTAB extraction buffer with 50% PEG and 1M NaCl **(for soil & sediment samples)**	***Work presented in this paper**		This work

TABLE 3: Spectrophotometric analysis of extracted DNA from different soil, sediment and water samples

(A)

Soil Sample	$A_{260/280}$ ratio	$A_{260/230}$ ratio	Conc. (μgmL^{-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. (μgmL^{-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. (μgmL^{-1})
S3 (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. (μgmL^{-1})
S4 (1)	1.808	1.651	9270
(2)	1.712	1.874	5100
(3)	1.646	1.854	3060

(E)

Sediment Sample	$A_{260/280}$ ratio	$A_{260/230}$ ratio	Conc. (μgmL^{-1})
SedTR (1)	1.795	1.780	5860
(2)	1.758	1.802	4580
(3)	1.612	1.821	3880

(F)

Sediment Sample	$A_{260/280}$ ratio	$A_{260/230}$ ratio	Conc. ($\mu g mL^{-1}$)
SedTM (1)	1.823	1.798	6020
(2)	1.720	1.814	5240
(3)	1.598	1.832	4080

(G)

Water Samples	$A_{260/280}$ ratio	$A_{260/230}$ ratio	Conc. ($\mu g mL^{-1}$)
WTR	1.804	1.902	4160
WTM	1.786	1.868	3220
WTP	1.818	1.894	3060

*Work presented in this paper

VI. Conclusion

Summarily, the soil and sediment DNA extraction and purification method described here, is a simple and efficient method for most of soil and sediment 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 and sediment 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.Also, data obtained from water DNA isolation showed an impressive potential of the standard Zhou et al protocol to be used for different water types (brackish water).

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