SDRP Journal of Earth Sciences & Environmental Studies(ISSN: 2472-6397)

Bacterial diversity assessment of different soil types from eastern states of India using 16S rDNA sequencing approach

Research

Author: Spandan Chaudhary

January 2018

Received Date: 14th Nov 2017
Accepted Date: 19th Dec 2017
Published Date:10th Jan 2018

***Spandan Chaudhary[1], Ravi Shaliwal[1], Pooja Chaudhary[1], Hiren Gajjar[1], Shiv Patel[1], Richa Misra[1], Pushprajsinh Chauhan[1], Jayesh Jalondhara[1], Harita Pandit[1] and Prashanth Bagali[1].**

[1]Xcelris Labs Limited, 2nd Floor, Heritage Profile, 22-23, Shrimali Society, Opp. Navrangpura Police Station, Navrangpura, Ahmedabad - 380009, Gujarat, INDIA

CORRESPONDENCE AUTHOR
Spandan Chaudhary
Email: spandan.chaudhary@xcelrislabs.com

CONFLICTS OF INTEREST
There are no conflicts of interest for any of the authors.

ABSTRACT
Soil is one of the reservoirs of the diverse group of microorganisms including bacteria, archeae, fungi etc., on the earth. Unculturable bacteria are of prime focus in scientific community because of their vital role in production of many important enzymes and degradation capabilities. Mostly, researcher all over the world are using NGS technologies to decode their genome which is costly when multiple isolates to be tested at initial level. In order to answer this question, we have tried gold standard Sanger sequencing technique to sequence metagenomic DNA extracted from soil. We used 16s rDNA approach and found microbes in the samples which are involved in processes such as Nitrogen fixation, oil-bioremediation, reduction of sulphate compounds, decomposition of aromatic compounds and hydrocarbons, removal of toxic metals etc., to maintain and increase sustainability of environment. Though, Sanger technique is widely used for sequencing of pure DNA, this is one of very few studies that have focused on metagenome diversity study using Sanger technology. This proves use of first generation technology as initial screening of metagenomic samples with desired microbes before going for comparatively expensive NGS sequencing.

KEYWORDS:
Soil DNA; Sanger sequencing; NGS; Metagenomic; unculturable bacteria

1.INTRODUCTION
Soil is one of the reservoirs of the diverse group of microorganisms including bacteria, archeae, fungi etc on the earth [1,2]. These microorganisms perform the key processes that create and maintain the environment in both terrestrial and aquatic systems. They also play an essential role in survival of other organisms on earth such as nitrogen fixing rhizobacteria in rhizospheric soil fix atmospheric nitrogen to make available it to the plants which utilize nitrogen as macro-nutrient, microorganisms which are found in the soil on polluted sites like sewage dumping site, wastelands, oil-contaminated sites etc. have the potential for conversion of complex organic and inorganic compounds in their simpler forms, thus, making their degradation easier and render them less harmful to environment as well as living beings on earth [3-6]. But this diversity of microorganisms in soil is still in its infancy in terms of accessibility because most of the soil microorganisms can't be cultured and isolated on standard solid or liquid media and by the current laboratory techniques because we lack critical information on their biology, and this presents both challenges and opportunities [7]. So, the hidden treasure of valuable biological information contained in genome of microorganisms is yet to be revealed because microbial diversity found in soil

environment is of immense importance regarding in the molecular aspects like identification and exploitation of specific microorganism for their genome which contains genes coding for products/processes characteristic to that microorganism. These products includes enzymes, antibiotics as well as other organic substances, bioremediation of polluted lands, removal of toxic metals(such as Chromium, Arsenic, Mercury etc.) and compounds, inhibition of pathogenic as well as harmful organism, development of treatment measures for diseases in plants as well as animals to increase productivity and yield. Microbial secondary metabolites are used in organ transplantation, cancer treatment and cholesterol control, as well as serving as insecticides, fungicides etc. Almost every aspect of human health would benefit from a greater diversity and availability of microbial natural products [8-12].

To obtain above advantage, the very first step is to isolate the microorganisms from soil environment and their further screening for information, we need. For this, One can use traditional biochemical tests [13] but the recent molecular biology techniques such as pulsed field gel electrophoresis(PFGE) of whole chromosomal DNA, RAPD & AFLP assays, 16S rDNA analysis, Real Time PCR and microarray based bacterial identification methods have been proved more promising [14-20]. All these methods has revolutionized the environmental microbiology [21]. Dominant species of microorganisms that best adapts to the ecosystem can be more prominently detected using these techniques as metabolically active microorganisms contain more DNA and RNA. In other words ecologically important microorganisms are assessed with molecular techniques and not the inactive ones which do not contribute to ecosystem functions. Another major advantage of these techniques is that microbial communities can be studied without actually cultivating the microorganisms thereby preserving the *in situ* metabolic status and composition. The new analytical approaches using DNA extracted directly from the environment enable us to access the genome, which is called metagenome, of all microorganisms inhabiting in the environment [22]. It made capable the researchers to develop effective and efficient culture-independent techniques for direct isolation and further screening and analysis of microbial diversity by using 16S rDNA methods provide the faster way to identify a specific group as well as diversity of microorganisms in soil and to correlate them to nearest species [23]. In addition to this, many researchers have been used and/or are using 16S rDNA sequences as a tool for taxonomic classification, usually where phenetic methods have proved lengthy and inconclusive [24].

In the present work, a modified protocol of CTAB-method for microbial genomic DNA isolation from different types of soils including agricultural, oil-contaminated, sewage, polluted water, desert soil etc., has been standardized and it is validated by analysis of microbial diversity in each type of extracted genomic DNA sample. The meta-genomic DNA was amplified by using the eubacterial primers designed from variable region of 16S rDNA sequences, the amplified PCR products were sequenced on ABI 3730XL.

2. MATERIALS AND METHODS

2.1 Site and sample description

Different types of soil samples were collected from different sites as mentioned in Table 1.

Table 1. Details of different types of soils with collection sites

Type of Soil	Collection Site	Location
Rhizospheric soil	Rhizospheric region of Jasmine Plant	Garden Near Xcelris Labs. Ltd, Ahmedabad
Cropland soil	lower part soil from chandan Plant	At Modasa, Gujarat
Cropland soil	Middle part of Bamboo Plant	At Modasa, Gujarat
Oil-rich soil	Petrol filling site	HP petrol pump at Mansi circle, Ahmadabad
Oil-contaminated soil	Petroleum waste dumping site	Indian Oil Corporation, Chandkheda, Ahmadabad
sandy soil	River bank site	Near Xcelris Labs. Ltd, Ahmedabad
Sewage soil	Urban solid and liquid waste(sewage) dumping area at Polluted River	¨Ayed¨ River at udaipur(Rajasthan)
Polluted water soil	Polluted area of water body	Fatehsagar Lake udaipur(Rajasthan)
Marshy soil	Polluted mine	Near chemical industries of Udaipur (Rajasthan)

2.2 Extraction of soil gDNA

Soil gDNA was extracted using modified CTAB protocol (TES-CTAB method) which consists of two phases (a) sample processing and (b) DNA isolation. Sample processing consist of washing of 0.5g to 5g weighed soil (depending upon soil type capable to form pellet after centrifuge) in 50ml1x Phosphate buffer, filtration of soil suspension with filter paper, centrifugation of filtrate at 4000 rpm for 20 minutes at 4°C pellet out the cells attached to soil particles, subsequently dissolving the pellet in to 4ml pre-warmed TES Buffer (100mM Tris, 10mM EDTA, 2% [w/v] sodium dodecyl sulphate, pH 8.0) and distributing it in four tubes. For DNA isolation one of the above tubes was taken and 10µl proteinase K (20mg/ml) is added and tube was incubated for 1 hour at 60°C. Further, 250µl 2% CTAB and 140µl 5M NaCl was added and incubated at 65° C for 20 minutes. 20µl RNase A (20mg/ml) was added in tube and incubated at 37° C for 45 minutes to remove RNA which usually precipitate with DNA. The lysed cells containing DNA in solution was divided into two tubes and mixed with equal volume of chloroform-isoamyl alcohol (24:1, v/v). The aqueous phase containing DNA was recovered by centrifugation at 14,000rpm at room temperature and precipitated with 0.1 volumes 3 M Sodium Acetate (pH-5.5) and 0.6 volume of isopropanol at -20°C for 1-2 hour or at -80°C for half an hour. Pellet of crude nucleic acid was obtained by centrifugation at 14,000rpm for 10 minutes, washed with cold 70 % ethanol and resuspended in sterile nuclease free water to a final volume of 30-50µl. These genomic DNA were quantified by Nanodrop 8000 (Thermoscientific) spectrophotometer. The purity of the extracted DNA was confirmed by running 1 to 2.5µg DNA separately from each sample on agarose gel electrophoresis set at 110V for 30-40 minutes at 25°C. The resultant DNA bands were visualized using Gel-Doc (Bio-Rad).

2.3 PCR Amplification of Extracted Soil DNA

DNA samples of each type of soil were amplified using eubacterial variable region 16S rRNA gene primers V_5F: 5'AAACTYAAARRATTGACGGG3' as forward primer and V_6R:5'CGACRRCCATGCANCACCT3' as reverse primer specific for Bacteria. The PCR amplification was carried out in Eppendorf Thermal cycler with 20 µl of final reaction volume containing 16.0µl DNase-RNase free water, 4µl 5XPCR reaction buffer (Roche),1.0µl DMSO, 1.0µl BSA, 0.02µl Taq DNA Polymerase mix(Roche), 1.0 µl forward primer V_5F, 1.0 µl reverse primer and 0.8 to 1.5µl diluted DNA (30ng/µl). The PCR was initiated with initial denaturation of DNA at 95°C for 5min and subsequently the number of cycles (94°C for 30s, 47°C for 30s and 72°C for 1min) were set to 35, and the final extension was performed at 72°C for 10min. 5 µl from the resulting PCR amplicons were mixed separately with 1µl of 6X gel loading dye and analyzed on 1.5% agarose gel containing ethidium bromide (0.1 µg/ml) at constant electric field of 5V/cm for 30min in 1X TAE buffer. The amplified PCR products of 16S rDNA variable region of sequences bacteria were confirmed as 200bp compact single band DNA visualized separately under UV-light using gel documentation system (Bio-Rad).

2.4 Sequencing and analysis of 16S rRNA gene sequences

The amplicons were purified with ExoSAP (USB) and subjected to automated DNA sequencing on ABI 3730xl Genetic Analyzer (Applied Biosystems, USA). Sequencing was carried out using Big Dye Terminator v3.1 Cycle sequencing kit following the manufacturer's protocol, where sequencing cycle was set with the thermal ramp rate of 1°C per second for 30 cycles (96°C for 5s; 47°C for 5s and 50°C for 4min). The resulted forward and reverse sequences of 16S rRNA genes of each type of soil sample were aligned with Codon Code aligner software and the consensus 16S rRNA gene sequences were obtained. These consensus gene sequences were used to identify the bacterial isolates with BLASTN analysis using NCBI GenBank Nr database. Based on maximum identity score twelve best 16S rRNA gene sequences were selected and aligned using multiple alignment software program ClustalW. The phylogentic tree was generated by neighbor-joining method using Mega v.4 software tool.

3. RESULTS AND DISCUSSION

3.1 DNA Extraction

In present study, it has been observed that modified CTAB protocol (TES-CTAB method) is effective to efficiently extract reasonably high molecular weight DNA from different types of soils with good yield which is also dependent upon soil type, pH, organic matter, clay and silt content as these factors can influence either the growth of certain microbial taxa, or the formation of aggregates with host microorganisms [21, 24, 25]. However, DNA yield is not only indicator of DNA extraction efficacy. Indeed, greater amounts of DNA do not necessarily mean that a greater number of Taxa can be detected. It is likely that extracted DNA mainly comes from easily lysed cells and aggregates [21, 26- 28], and therefore, differences in microbial cell wall structure and micro habitats will affect the extraction of DNA and thus analyses of diversity. Quantification of Extracted DNA was carried out using Nanodrop spectrophotometer (Table 2). The quality and purity of these DNA samples were further confirmed by agarose gel (0.8%) electrophoresis resulting in the single band of high molecular weight DNA under UV illumination (Figure 1).

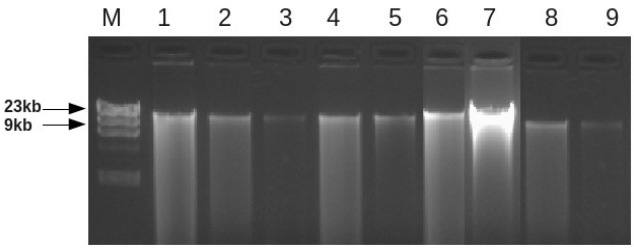

Figure 1: Agarose gel (0.8%) electrophoretogram of the total DNA extracted from different types of soils. 1-Rhizospheric Soil, 2-Cropland soil(Bamboo), 3-Cropland soil(chandan), 4-oil-rich soil, 5-Oil-Contaminated Soil, 6-sandy Soil (Sand), 7-Sewage Soil, 8-Lake Soil, 9-Marshy soil, M-HINDIII DNA ladder.

3.2 PCR Amplification and Sequencing Analysis

The PCR was performed using Eubacterial primers for Variable 16S rRNA region V5 and V6 and resulting PCR amplicons were visualized as a single intact band of expected size 160-180bp DNA using 1.5% agarose gel electrophoresis (Figure 2). Bacterial diversity was detected in phylogentic tree for 16S rRNA sequence of each type of soil sample and found that most of them are uncultured bacteria. Bacterial Community was generally represented by *Protobacteria*, *Acidobacteria, Fermicutes, Bacteriodetes*.

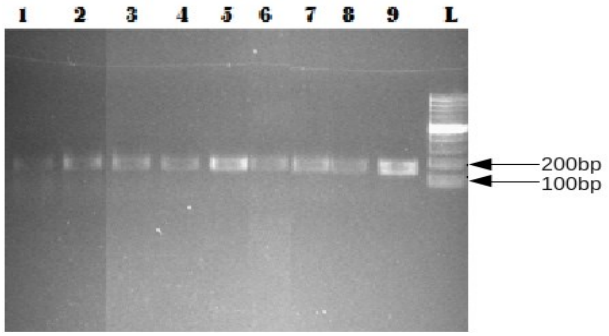

Figure 2: Agarose gel(1.5%) electrophoretogram of PCR Amplfied 16S rRNA gene of extracted DNA from Different types of soils. 1- Cropland soil(chandan), 2-Cropland soil(Bamboo), 3- Rhizospheric Soil 4- -sandy Soil (Sand), 5- oil-rich soil, 6-Marshy soil, 7-Sewage Soil, 8-Polluted water Soil, 9-Oil-Contaminated Soil, L -100bp DNA ladder

The homologous organisms for bacterial community present in crop land soil (Bamboo as well as Chandan) as shown in the Table 3 & 4 where maximum similarity (80%-91%) was found to the genus *Pseudomonas* that were uncultured. Majority of these uncultured strains belonged to the phyla Delta-Protobacteria. However, most probable nearest neighborhood strain may be considered as the, *Coriobacteriaceae* bacterium clone Pad-127(JX505374.1) and Uncultured organism clone SBZP_5567 (JN538754.1) for bacterial community present in Bamboo and Chandan Soil, respectively which is also evident from the phylogenetic tree shown in the Figure 3 & 4. Similarly, bacterial strains present in Rhizosheric soil also showed homology with uncultured strains of diverse type viz forest soil bacterium, *Actinobacterium, Rubrobacteridae* bacterium, *Solirubrobacter sp. Clone, Gemmatimonadetes* bacterium in which nearest neighborhood strain is *Marinobacter flavimaris strain* SDT4S11 (JQ068802.1) that is reported as halophilic, hydrocarbonoclastics bacterium with diazotrophic potential (Figure 5) majority of them are found in hypersaline waters and soils. Experimental evidence suggests their nitrogen-fixation potential [29]. Strains of this species can successfully mineralized crude oil in nutrient media as well as in hypersaline soil or water microcosms without the use of any nitrogen fertilizers.

Table2. Nanodrop readings of Extracted DNA from Different types of soil

S.N.	Soil type	$A_{260/280}$	DNA yield (ng/µl)
1	Rhizospheric soil	1.34	41.87
2	Cropland soil (Bamboo)	1.25	96.66
3	Cropland soil(Chandan)	1.25	50.63
4	Oil rich soil	1.65	30.63
5	Oil contaminated soil	1.60	31.21
6	Desert (sandy) soil	1.39	15.71
7	Sewage soil	1.64	649.8
8	Polluted water	1.42	69.29
9	Marshy soil	1.24	38.72

In case of oil rich and oil contaminated soil, Phylogentic tree (Figure 6 & 11) revealed that uncultured strains are predominant but somewhat different strains in both type of soil like uncultured bacterium clone EMIRGE_OTU_s6b4a_7194 (JX224145.1) and uncultured *Sphingobacteriales* bacterium clone GE7GX-PU01A91FX(HM975819.1) which are nearest neighbor of bacterial strains present in oil-rich soil and also shows the closeness with each other whereas uncultured bacterium clone SM2F31(EU879395.1) and uncultured bacterium clone nbw775c10c1(GQ009344.1) are the nearest neighbor of bacterial strains in oil-contaminated soil with maximum similarity (80-81%). Besides this *Sphingobacteriales, Rikenellaceae, Desulfobulbus and Protobacteria, Actinobacteria, Stenotrophomonas, Lysobacter, Pseudomans* also showed homology with bacterial strains present in both type of

soil (Table 6 & 11).

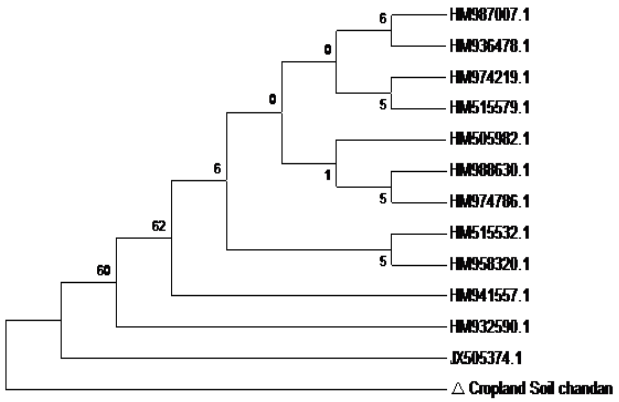

Figure 3: Phylogenetic tree of Bacterial strains present in Cropland Soil (chandan) soil with selected best homologous known strains

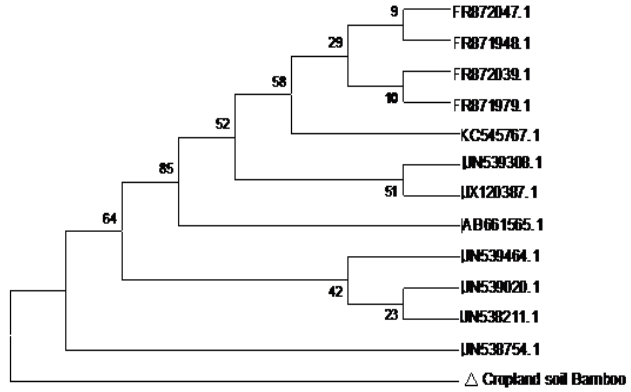

Figure 4: Phylogenetic tree of bacterial strains present in cropland soil (Bamboo) soil with selected best homologous known strains

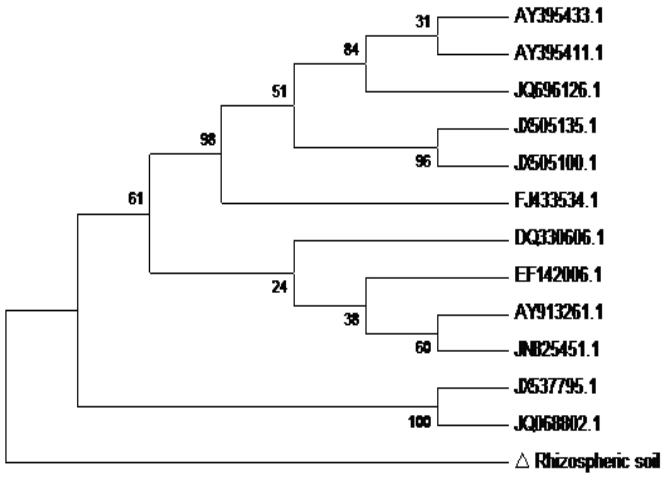

Figure 5: Phylogenetic tree of bacterial strains present in rhizosheric soil with selected best homologous known strain

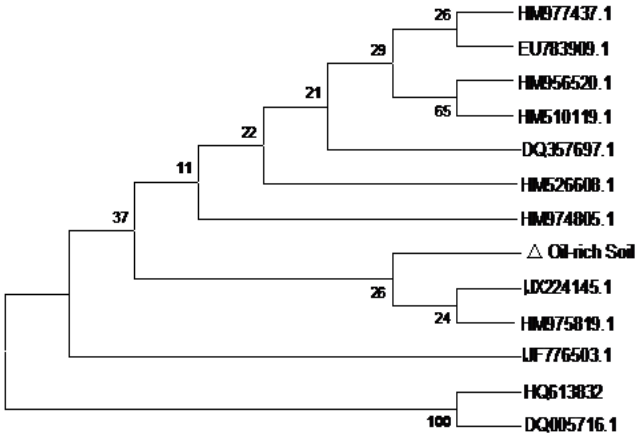

Figure 6: Phylogenetic tree of bacterial strains present in oil-rich soil with selected best homologous known strains

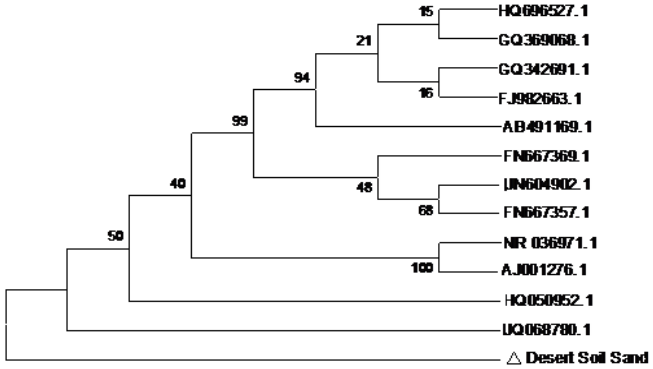

Figure 7: Phylogenetic tree of bacterial strains present in sandy soil (sand) with selected best homologous known strains

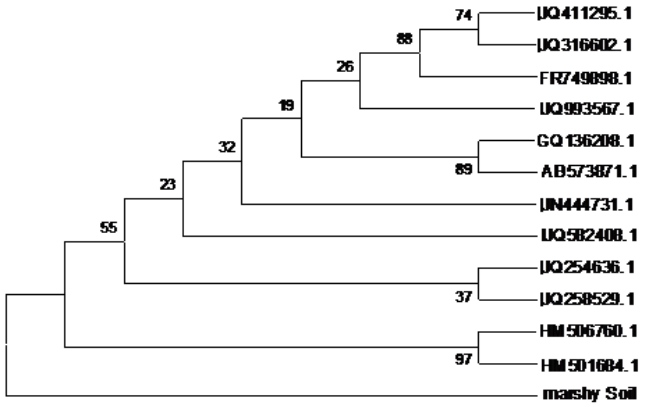

Figure 8: Phylogenetic tree of bacterial strains present in marshy soil with selected best homologous known strain

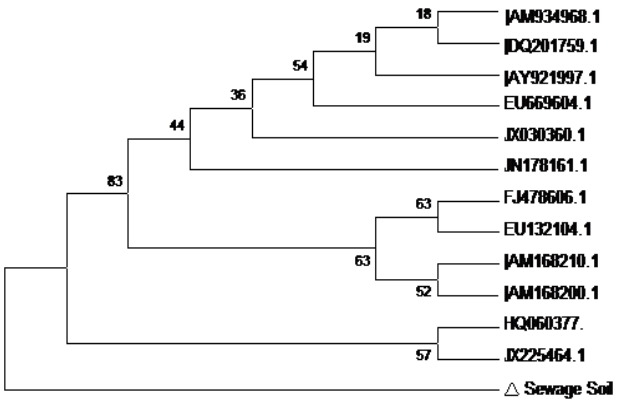

Figure 9: Phylogenetic tree of bacterial strains present in sewage soil with selected best homologous known strains

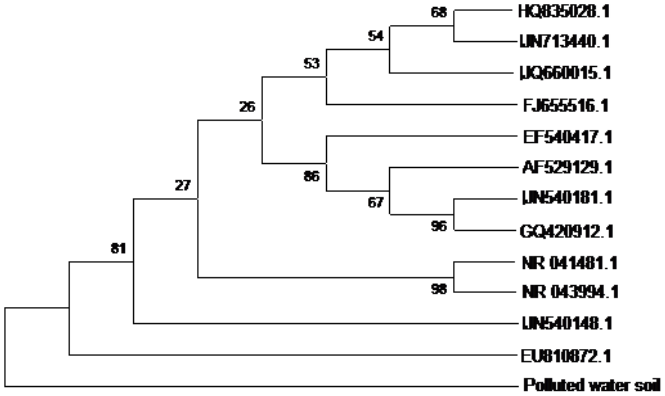

Figure10: Phylogenetic tree of bacterial strains present in polluted water soil with selected best homologous known strains

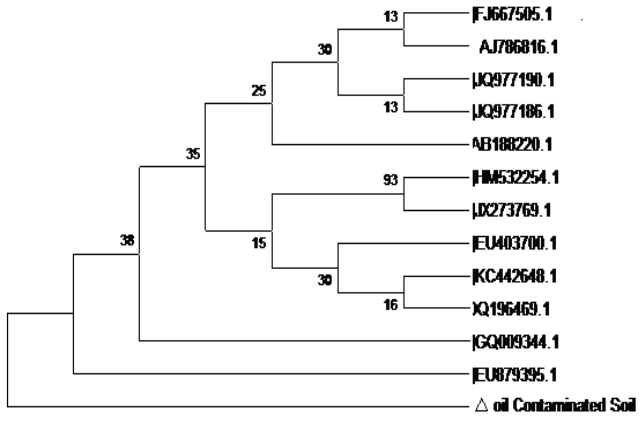

Figure11. Phylogenetic tree of bacterial strains present in oil contaminated soil with selected best homologous known strains.

Phylogenetic tree of bacterial strains present in Desert Soil (Sand) shows homology with uncultured bacterial strains as well as many known strains such as *Rhodospira trueperi strain ATCC 700224* (AJ001276.1), *Brevibacillus agri strain ABRII11* (JN604902.1), *Brevibacillus borstelensis strain: AHK190* (AB491169.1)*, Brevibacillus thermoruber strain T1SS10* (GQ342691.1) with maximum similarity (77%-78%). But uncultured *Syntrophaceae* bacterium clone F5oHPNU07H3PTE (HQ050952) and *Nitrosococcus oceani* strain SDT3S16 (JQ068780.1) are nearest neighbor of bacterial strains present in Desert Soil (Sand). Among these, *Nitrosococcus oceani* is a member of the evolutionary oldest taxonomic group capable of lithotrophic ammonia catabolism. The gammaproteobacterium *Nitrosococcus oceani* (ATCC 19707) is a gram-negative obligate chemolithoautotroph capable of extracting energy and reducing power from the oxidation of ammonia to nitrite [30].

Uncultured *Desulfobulbus sp.* clone GE7GXPU01CGYJL (HM506760.1) and uncultured *Desulfobulbus sp.* clone GE7GXPU01B52YQ (HM501684.1) were found to be the nearest neighbor of the bacterial community present in marshy soil with maximum similarity of 83%. The genus *Desulfobulbus* (which is placed under class *Deltaprotobacteria*) have been studied earlier as sulphate reducing bacteria (SRB) found in the anaerobic sediments at eutrophicated sites polluted with heavy metals, particularly with mercury [31]. It has been also reported that SRB can destroy organic pollutants and can bind heavy metal ions from solutions to non-soluble sulfides. Many toxic metals like cadmium, mercury, tin, zinc, nickel, cobalt, gold, silver and uranium were found in reservoirs are known to have a toxic effects. Conversely, Acidobacteria, Unidentified soil bacteria and uncultured bacterial strains have shown homology to bacterial community present in sewage soil with maximum similarity of 85%-89%. But uncultured *Syntrophorhabdus sp.* clone F5OHPNU07HX3UX (HQ060377.1) and uncultured bacterium clone EMIRGE_OTU_s8b4e_473 (JX225464.1) were showing the nearest neighborhood with bacterial community present in sewage soil as evident from phylogenetic tree (Figure 9). Peripheral 16S rRNA gene sequences in the databases indicated that the proposed new family Syntrophorhabdaceae is largely represented by abundant bacteria within anaerobic ecosystems mainly decomposing aromatic compounds [32]. The polluted water soil have diverse bacterial community which were showed homology with various types of bacteria such as *Geobacter sp., Luteimonas sp.*, *Xanthomonas sp.*, *Desulfuromonas sp.*, secondary symbiont of *Stomaphis quercus* etc but the nearest neighbor was found to be Actinobacterium01QJ5 (EU810872.1), uncultured *Syntrophobacterales* bacterium clone Agri_anode1_191 (JN540148.1). It has been reported that *Actino bacteria* include some of the most common soil life, freshwater

life, and marine life, playing an important role in the decomposition of organic materials, such as cellulose and chitin, and thereby playing a vital part in organic matter turnover and the carbon cycle. In the soil, this replenishes the supply of nutrients and is an important part of humus formation whereas genus *Syntrophobacter* consists of rod-shaped bacteria growing in syntrophic association with hydrogen- and formate-scavenging microorganisms. Many of the *Syntrophobacter* spp. is able to use sulfate as the electron acceptor for propionate oxidation and some other organic compounds and hydrogen. The other nearest could be *Deinococcus roseus strain* TDMA-uv51 (NR_041481.1) and *Deinococcus cellulosilyticus* strain 5516J-15 (NR_043994.1). These bacteria have thick cell walls that give them gram-positive stains but they include a second membrane and so are closer in structure to those of gram-negative bacteria. They are also characterized by the presence of the carotenoid pigment Deinoxhantin that give them their pink color, and a high resistance to gamma and UV radiation and are usually isolated according to these two criteria. The first one is gamma- and UV-radiation resistant, Gram-positive, red- or pink-pigmented, rod-shaped, strictly aerobic, oxidase- and catalase-positive bacterial strain, was isolated from fresh water collected at Misasa, a radioactive site in Japan [33]. Phylogenetic analysis based on 16S rRNA gene sequences placed it in a distinct lineage in the family *Deinococcaceae*, along with another similar strain TDMA-25T.The strains degraded gelatin, casein, starch and Tween 80. Unique physiological characteristics, differences in their fatty acid profiles, and genotypic and phylogenetic features, differentiated strains TDMA-25T and TDMA-uv51T from closely related Deinococcus species. Hence, the two strains are described as novel species of the genus Deinococcus. The names *Deinococcus misasensis sp.* nov. (type strain TDMA-25T=JCM 14369=NBRC 102116=CCUG 53610) and *Deinococcus roseus sp.* nov. (type strain TDMA-uv51T=JCM 14370=NBRC 102117=CCUG 53611) are proposed [33].

Table 3. Bacterial strains present in cropland soil (chandan) homology to nearest known neighborhood bacterial strains

Accession No.	Bacterial strain	Query Coverage	E-Value	Max Identity
HM515532.1	Uncultured Pseudomonas sp. clone GE7GXPU01C20DQ	35%	2e-07	90%
HM988630.1	Uncultured Pseudomonas sp. clone GE7GXPU01D2EIG	33%	2e-06	91%
HM987007.1	Uncultured Pseudomonas sp. clone GE7GXPU01BZBNY	35%	2e-06	90%
HM974786.1	Uncultured Pseudomonas sp. clone GE7GXPU01EF96B	33%	2e-06	91%
HM974219.1	Uncultured Pseudomonas sp. clone GE7GXPU01BNMFN	35%	2e-06	90%
HM958320.1	Uncultured Pseudomonas sp. clone GG5QJA201BAX5J	35%	2e-06	90%
HM941557.1	Uncultured Pseudomonas sp. clone GG5QJA201AQIU8	33%	2e-06	91%
HM936478.1	Uncultured Pseudomonas sp. clone GG5QJA201A1EA6	33%	2e-06	91%
HM515579.1	Uncultured Pseudomonas sp. clone GE7GXPU01A1TXG	33%	2e-06	91%
HM505982.1	Uncultured Pseudomonas sp. clone GE7GXPU01ASZ9I	35%	2e-06	90%
HM932590.1	Uncultured Pseudomonas sp. clone GG5QJA201AGDWS	35%	2e-06	91%
JX505374.1	Uncultured Coriobacteriaceae bacterium clone Pad-127	35%	3e-05	88%

Table 4. Bacterial strains present in cropland soil (Bamboo) homology to nearest known neighborhood bacterial strains

Accession No.	Bacterial strain	Query Coverage	E-Value	Max Identity
KC545767.1	Uncultured candidate division WS3 bacterium clone BFB087	51%	3e-04	70%
FR872047.1	Uncultured bacterium partial 16S rRNA gene, clone GoM_Bac_71	51%	3e-04	80%
FR872039.1	Uncultured bacterium partial 16S rRNA gene, clone GoM_Bac_5	51%	3e-04	80%
FR871979.1	Uncultured delta proteobacterium clone GoM_Bac_5	51%	3e-04	80%
FR871948.1	Uncultured delta proteobacterium clone GoM_Bac_71	51%	3e-04	80%
JX120387.1	Uncultured bacterium clone UA_17	51%	3e-04	80%
AB661565.1	Uncultured bacterium clone: B60	51%	3e-04	80%
JN539464.1	Uncultured organism clone SBZP_6329 16S ribosomal RNA gene, partial sequence	51%	3e-04	80%
JN539308.1	Uncultured organism clone SBZP_6160	51%	3e-04	80%
JN539020.1	Uncultured organism clone SBZP_5855	51%	3e-04	80%
JN538754.1	Uncultured organism clone SBZP_5567	51%	3e-04	80%
JN538211.1	Uncultured organism clone SBZP_4961	51%	3e-04	80%

Table 5. Bacterial strains present in rhizospheric soil homology to nearest known neighborhood bacterial strains

Accession No.	Bacterial strain	Query Coverage	E-Value	Max Identity
AY913261.1	Uncultured forest soil bacterium clone DUNssu041	51%	4e-10	87%
JQ696126.1	Uncultured bacterium clone 4783619	51%	1e-09	85%
FJ433534.1	Uncultured bacterium clone YG-D1210	51%	1e-09	85%
EF142006.1	Uncultured actinobacterium clone KF028	51%	1e-09	85%
DQ330606.1	Uncultured candidate division GN03 bacterium clone 05D214B	52%	1e-09	85%
AY395433.1	Uncultured Rubrobacteridae bacterium clone EB1114	54%	1e-09	85%
AY395411.1	Uncultured Rubrobacteridae bacterium clone EB1092	51%	1e-09	85%
JX505135.1	Uncultured Solirubrobacter sp. clone D.an-68	51%	4e-09	85%
JX505100.1	Uncultured Solirubrobacter sp. clone D.an-33	50%	4e-09	85%
JX537795.1	Marinobacter sp. Anaero4	50%	4e-09	86%
JQ068802.1	Marinobacter flavimaris strain SDT4S11	49%	4e-09	86%
JN825451.1	Uncultured Gemmatimonadetes bacterium clone Alchichica_AQ1_1_1B	59%	4e-09	85%

Table 6. Bacterial strains present in oil-rich soil homology to nearest known neighborhood bacterial strains

Accession No.	Bacterial strain	Query Coverage	E-Value	Max Identity
JX224145.1	Uncultured bacterium clone EMIRGE_OTU_s6b4a_7194	85%	1e-30	88%
HM974805.1	Uncultured Pseudomonas sp. clone GE7GX-PU01BDX6S	90%	3e-30	86%
HM975819.1	Uncultured Sphingobacteriales bacterium clone GE7GX-PU01A91FX	90%	1e-29	87%
JF776503.1	Lysobacter sp. DJM4C11	91%	1e-28	84%
HQ613832.1	Pseudomonas sp. BND-BHI2	90%	1e-28	85%
HM977437.1	Uncultured Pseudomonas sp. clone GE7GX-PU01DPPMH	90%	1e-28	85%
HM956520.1	Uncultured Rikenellaceae bacterium clone GG5QJA201EZ0MZ	85%	1e-28	86%
HM526608.1	Uncultured Desulfobulbus sp. clone GE7GX-PU01CP10K	90%	1e-28	85%
HM510119.1	Uncultured Sphingobacteriales bacterium clone GE7GXPU01B9LWU	92%	1e-28	85%
EU783909.1	Pseudomonas sp. hs2	92%	1e-28	85%
DQ357697.1	Pseudomonas sp. Sc-R8 clone 420.1	85%	1e-28	86%
DQ005716.1	Acinetobacter sp. ST-FER-2	90	2e-26	84%

Table 7. Bacterial strains present in sandy soil homology to nearest known neighborhood bacterial

Accession No.	Bacterial strain	Query Coverage	E-Value	Max Identity
NR036971.1	Rhodospira trueperi strain 8316	75%	2e-09	78%
AJ001276.1	Rhodospira trueperi strain ATCC 700224	75%	2e-09	78%
JQ068780.1	Nitrosococcus oceani strain SDT3S16	75%	2e-08	76%
JN604902.1	Brevibacillus agri strain ABRII11	76%	2e-08	77%
HQ696527.1	Actinobacterium MH6	76%	2e-08	78%
HQ050952.1	Uncultured Syntrophaceae bacterium clone F5OHPNU07H3PTE	75%	2e-08	77%
GQ369068.1	Brevibacillus sp. Z0-YC6800	76%	2e-08	78%
FN667369.1	Uncultured compost bacterium clone PS2573	76%	2e-08	78%
FN667357.1	Uncultured compost bacterium clone PS2528	76%	2e-08	78%
AB491169.1	Brevibacillus borstelensis strain: AHK190	76%	2e-08	78%
GQ342691.1	Brevibacillus thermoruber strain T1SS10	76%	2e-08	78%
FJ982663.1	Brevibacillus borstelensis strain JBE0014	76%	2e-09	78%

Table 8. Bacterial strains present in marshy soil homology to nearest known neighborhood bacterial

Accession No.	Bacterial strain	Query Cov-erage	E-Value	Max Identity
GQ136208.1	Uncultured bacterium clone 09c07	71%	2e-07	84%
JN444731.1	Uncultured organism clone SBYC_533	71%	2e-07	84%
JQ993567.1	Uncultured Desulfovibrio sp. clone ImrTc_5	76%	3e-06	84%
JQ582408.1	Desulfonatronovibrio sp. SLSR1	69%	3e-06	84%
JQ254636.1	Uncultured Xanthomonadales bacterium clone 5-3-p1-P	77%	3e-06	83%
JQ411295.1	Desulfovibrio sp. P23	69%	3e-06	84%
JQ316602.1	Uncultured bacterium clone WRa01	69%	3e-06	84%
JQ258529.1|	Uncultured bacterium clone M-UB-68	70%	3e-06	83%
FR749898.1	Desulfovibrio giganteus type strain DSM 4123T	69%	3e-06	84%
AB573871.1	Desulfomicrobium orale strain: JCM 17150	69%	3e-06	84%
HM506760.1	Uncultured Desulfobulbus sp. clone GE7GX-PU01CGYJL	71%	3e-06	83%
HM501684.1	Uncultured Desulfobulbus sp. clone GE7GX-PU01B52YQ	70%	3e-06	83%

Table 9. Bacterial strains present in s*ewage soil* homology to nearest known neighborhood bacterial strains

Accession No.	Bacterial strain	Query Coverage	E-Value	Max Identity
JN178161.1	Uncultured bacterium clone TX2_4A17	37%	2e-07	89%
HQ060377.1	Uncultured Syntrophorhabdus sp. clone F5OHPNU07HX3UX	41%	2e-07	89%
JX030360.1	Uncultured Candidatus Chloracido-bacterium sp. clone C-20	45%	3e-06	85%
FJ478606.1	Uncultured bacterium clone p11m04ok	45%	3e-06	85%
EU669604.1	Uncultured bacterium clone S11-26	45%	3e-06	85%
EU132104.1	Uncultured bacterium clone FFCH13830	45%	3e-06	85%
AM168210.1	Unidentified soil bacteria	45%	3e-06	85%
AM168200.1	Unidentified soil bacteria clone 69	37%	3e-06	85%
DQ201759.1	Uncultured Acidobacteria bacterium clone Oi15	45%	3e-06	89%
AY921997.1	Uncultured Acidobacteria bacterium clone AKYG1861	45%%	3e-06	85%
JX225464.1	Uncultured bacterium clone EMIRGE_OTU_s8b4e_473	38%	1e-04	85%
AM934968.1	Uncultured Acidobacteriaceae bacte-rium clone AMLE11	45%	3e-06	85%

Table10. Bacterial strains present in polluted water homology to nearest known neighborhood bacterial

Accession No.	Bacterial strain	Query Coverage	E-Value	Max Identity
EU810872.1	Actinobacterium 01QJ5	70%	3e-12	79%
HQ835028.1	Uncultured bacterium clone So-62	88%	1e-11	75%
JN713440.1	Luteimonas sp. canine oral taxon 275 clone ZN009	80%	1e-10	76%
JQ660015.1	Xanthomonas sp. R9-740	88%	1e-09	75%
JN540181.1	Uncultured Desulfuromonadales bacterium clone Agri_anode1_154	88%	1e-09	76%
JN540148.1	Uncultured Syntrophobacterales bacterium clone Agri_anode1_191	88%	1e-09	76%
GQ420912.1	Uncultured Geobacter sp. clone RUGL1-418	88%	1e-09	76%
EF540417.1	Uncultured soil bacterium clone MK42	81%	1e-09	75%
NR_041481.1	Deinococcus roseus strain TDMA-uv51	80%	1e-09	76%
F529129.1	AUncultured delta proteobacterium clone FTLpost101	88%%	1e-09	76%
FJ655516.1	Secondary symbiont of Stomaphis quercus	88%	5e-09	74%
NR_043994.1	Deinococcus cellulosilyticus strain 5516J-15	80%	5e-09	77%

Table11. Bacterial strains present in oil contaminated soil homology to nearest known neighborhood bacterial strains

Accession No.	Bacterial strain	Query Coverage	E-Value	Max Identity
AB188220.1	Luteimonas sp. TUT1238	89%	1e-24	82%
FJ667505.1	Pseudomonas sp. ZZ-7	89%	3e-24	82%
EU879395.1	Uncultured bacterium clone SM2F31	93%	3e-24	81%
KC442648.1	Uncultured proteobacterium clone 3B12	89%	4e-23	81%
GQ009344.1	Uncultured bacterium clone nbw775c10c1	93%	4e-23	80%
EU403700.1	Uncultured Xanthomonadales bacterium clone MP10B17	89%	4e-23	81%
DQ196469.1	Xanthomonas sp. L60	89%	4e-23	81%
HM532254.1	Uncultured Stenotrophomonas sp. clone GE7GXPU01D8UZV	89%	1e-22	81%
AJ786816.1	Unidentified bacterium isolate R-23043	89%	1e-22	81%
JQ977190.1	Xanthomonas sp. Gra17	89%	5e-22	81%
JQ977186.1	Lysobacter sp. Gra9	89%	5e-22	81%
JX273769.1	Stenotrophomonas maltophilia strain M9	89%	5e-22	81%

CONCLUSION

The results presented here demonstrate that the Sanger sequencing method can be used for initial screening of diversity samples before going for high throughput data generation. Standard practice for diversity studies includes DNA isolation from natural samples and subsequently their sequencing by advanced sequencing platforms using 16S rDNA approaches but present study has provided the evidences that Sanger sequencing can also be used for small scale diversity studies.

ACKNOWLEDGMENTS

Authors like to thank management of Xcelris Labs Ltd. for providing infrastructure and facility to conduct the study.

AUTHOR CONTRIBUTION

Spandan Chaudhary - Designing and mentoring of experiment, Writing and Editing of manuscript, Prashanth Bagali - Mentoring the whole project, Ravi Shaliwal- Sample collection and draft manuscript preparation, Pooja Chaudhary - Sequencing of samples, Richa, Pushparaj, Jayesh, Harita and Shiv - Sample collection, DNA isolation and 16s analysis

REFERENCES

1. P.A. Maron, C. Mougel, L. Ranjard, Soil microbial diversity: Methodological strategy, special and functional interest, C R Biol (2011) 334: 403-411.
2. P. Robe, R. Nalin, C. Capellano, T.M. Vogel, P. Simonet, Extraction of DNA from soil, Eur J Soil Biol (2003)39: 183-190.
3. R. Rynk, M.V.D. Kamp, G.B. Willson, M.E. Singley, T.L. Rechard, J.L. Kolega, Gouin FR, Laliberty L, On Farm Composting Handbook. New York, Cornell University 1992.
4. W. Borken , A. Muhs, and F. Reese, Chanches in microbial and soil properties following compost treatment of degraded temperate forest soils, Soil Biol Biochem (2002) 34; 403-412.
5. B. Beck-Friis, S. Smårs, H. Jönsson, Y. Eklind, and H. Kirchmann, Composting of source-separated household organics at different oxygen levels: Gaining an understanding of the emission dynamics, Compost Science & Utilization 11, no. 1 (2003): 41-50.
6. R.B. Srivastava, A. Bora, Microbial means of biowaste management, IJBST. 2009;2(4):52-61.
7. E. J. Stewart, Growing Unculturable Bacteria. American Society for Microbiology J. Bacteriol (2012) 194(16), pp.4151-4160. doi:10.1128/JB.00345-12
8. A.L. Demain and S. Sanchez, Microbial drug discovery: 80 years of progress. J Antibiot (Tokyo) (2009) 62:5-16.
9. M.K. Swamy. S.S.N Kashyap, R. Vijay, T. Tiwari, M. Anuradha M. Production and optimization of extra cellular protease from Bacillus sp. isolated from soil. Int J Adv Biotech Res (2012); 3(2):564-569.
10. S.O. Ramchuran, V.A. Vargas, R. Hatti-Kaul, E.N. Karlsson, Production of a lipolytic enzyme originating from Bacillus halodurans LBB2 in the methylotrophic yeast Pichia pastoris, Applied microbiology and biotechnology (2006) 1;71(4):463-72.
11. A. Sridevi, G. Narasimha, B.R. Reddy, Production of cellulases by Aspergillusniger on natural and pretreated lignocellulosic waste. Int. J. Microbiol. (2009) 7(1).
12. S. Shafique, M. Asgher, M.A. Sheikh, M.J. Asad, Solid state fermentation of banana stalk for exoglucanase production. International Journal of Agriculture and Biology (2004)3:488-91.
13. Karbaum K. Bergey's manual of systematic bacteriology–Volume 2. baltimore, London, Los Angeles, Sidney: Williams and Wilkins (1986) pp. 965–1599, 75$.
14. Kostman JR, Edlin TD, Lipuma JL, Stull TL. Molecular epidemiology of Pseudomonas cepacia determined by polymerase chain reaction ribotyping. J Clin Microbiol (1992) 30:2084–2087.
15. P.J. Villadas, P. Burgos, D. Jording, W. Selbitschka, A. Pühler, N. Toro, Comparative analysis of the genetic structure of a Rhizobium meliloti field population before and after environmental release of the highly competitive R. meliloti strain GR4. FEMS microbiology ecology (1996)21(1):37-45.
16. J. Welsh, J. and M. McClelland, Fingerprinting genomes using PCR with arbitrary primers. Nucleic acids research (1990) 18(24), pp.7213-7218.
17. J.G.Williams, A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey, DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic acids research (1990) 18(22), pp.6531-6535.
18. J.A. Robertson, A. Vekris, C. Bebear, and G.W. Stemke, Polymerase chain reaction using 16S rRNA gene sequences distinguishes the two biovars of Ureaplasma urealyticum. Journal of clinical microbiology (1993) 31(4), pp.824-830.
19. D.M. Olive, P. Bean, Principles and applications of methods for DNA-based typing of microbial organisms. Journal of clinical microbiology (1999) Jun 1;37(6):1661-9.
20. B. Mandepudi, D. Mandepudi, V.C. Ghanta, Identification and characterization of novel lipase producing soil bacterial isolates B3 and B4 using 16S rDNA analysis. Res. J. Pharm. Biol. Chem. Sci. (2013)4:149-60.
21. T.O. Deimont, P. Robe, I. Clarck, Simonet, T.M. Vogel, Metagenomic comprision of direct and soil DNA extraction approaches. J Microbiol Methods (2011) 86: 397-400.

22. Y. Pan, L. Bodrossy, P. Frenzel, A.G. Hestnes, S. Krause, C. Lüke, M. Meima-Franke, H. Siljanen, M.M. Svenning, P.L. Bodelier, Impacts of inter-and intralaboratory variations on the reproducibility of microbial community analyses. Applied and environmental microbiology (2010) 15;76(22):7451-8.
23. A. Macrae, The use of 16S rDNA methods in soil microbiologyse . Brazilian Journal of Microbiology (2000) 31:77-82
24. J. Zhou, M.A. Burns, J.M. Tiedje, DNA recovery from soils of diverse compositions. Appl Enviorn Microbiol (1996) 62: 316-322
25. N. Fortin, D. Beaumier, K. Lee, C. W. Greer, Soil washing improves the recovery of total community DNA from polluted and high organic content sediments. J Microbiol Methods (2004) 56: 181-191.
26. F.M. Lakay, A. Botha, B.A. Prior, Comparative analysis of environmental DNA extraction and purification methods from different humic acid-rich soil Journal of applied microbiology (2007)102 (1):265-73.
27. C.L. Roose-Amsaleg, E. Garnier-sillam, M. Harry, Extraction and purification of microbial DNA from soil and Sediment Samples. Agric, Ecosyst Enviorn, Appl Soil Ecol (2001) 18: 47-60.
28. J.E.M. Stach, S. Bathe, J.P. Clapp, R.G. Burns, PCR-SSCP comparison of 16s rDNA sequence diversity in soil DNA obtained using different isolation and purification methods. FEMS Microbiol Ecol (2001) 36: 139-151.
29. B.B.Ward, G.D. O'Mullan, Worldwide distribution of Nitrosococcus oceani, a marine ammonia-oxidizing Y-proteobacterium, detected by PCR and sequencing of 16S rRNA and amoA genes. Applied and environmental microbiology (2002) Aug 1;68 (8):4153-7.
30. M.G.Klotz, D.J. Arp, P.S. Chain, A.F. El-Sheikh, L.J. Hauser, N.G.Hommes, F.W. Larimer, S.A.Malfatti, J.M. Norton, A.T. Poret-Peterson, L.M. Vergez, Complete genome sequence of the marine, chemolithoautotrophic, ammonia-oxidizing bacterium Nitrosococcus oceani ATCC 19707. Applied and environmental microbiology (2006) 72(9):6299-315.
31. K.S. Fishman, V.N. Akimov, N.E. Suzina, M.B. Vainshtein, X. Liang. Sulfate-reducing bacteria Desulfobulbus sp. strain BH from a freshwater lake in Guizhou Province, China. Inland water biology (2013) 6(1):13-7.
32. Y.L.Qiu, S.Hanada, A. Ohashi, H. Harada, Y. Kamagata, Y. Sekiguchi, Syntrophorhabdus aromaticivorans gen. nov., sp. nov., the first cultured anaerobe capable of degrading phenol to acetate in obligate syntrophic associations with a hydrogenotrophic methanogen. Applied and environmental microbiology (2008) Apr 1;74(7):2051-8.
33. D. Asker, T.S. Awad, T. Beppu, K. Ueda, Deinococcus misasensis and Deinococcus roseus, novel members of the genus Deinococcus, isolated from a radioactive site in Japan. Systematic and applied microbiology (2008) 19;31(1):43-9.

Contact Us:
SIFT DESK
Deerpark Dr, #75, Fullerton,CA,92831,United States.
E-mail: helpdesk@siftdesk.org
Visit us on the web at: **www.siftdesk.org**