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Research Article

METAGENOMIC ANALYSIS OF BACTERIAL COMMUNITY IN SOIL USING 16S rRNA GENE THROUGH HIGH THROUGHPUT SEQUENCING

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Abstract- Bacterial 16S ribosomal DNA (rDNA) amplicons have been widely used in the classification of uncultured bacteria inhabiting environmental niches. Primers targeting conservative regions of the rDNAs are used to generate amplicons for metagenomics study. The present study applied metagenomics to characterize the diversity and relative occurrence of Bacterial organisms in the soils of Chandan plant (MS1) and Bamboo plant (MS2) using high throughput sequencing. The metagenome samples were subjected to sequencing by Ion torrent PGM which resulted in 1,418,770 (MS1) and 1,695,228 (MS2) reads respectively. The taxonomic profile obtained by comparison with M5NR database showed predominance of *Actinobacteria, Proteobacteria* and *Firmicutes* in both the samples, abundance of Actinobacteria in MS-1(46.84%) and in MS-2(47.06%), followed by Proteobacteria in MS-1(26.80%) and in MS-2(26.91%) , *Firmicutes* in MS-1(23.11%) and MS-2(22.79%). At genus level, metagenomics revealed 16 genera in soil 1 (MS1) and 18 genera in soil 2 (MS2), *Mycobacterium* is predominant in both the samples, in MS-1(33.18%) and in MS-2 (32.97%). The present study provides a preliminary snapshot of the diversity and relative abundance of the bacteria within the soil samples and expands our knowledge of these dynamic Bacterial communities present in the soil ecosystem and these consortiums may be helpful in soil fertility and enhance plant productivity.

Keywords- Soil, 16S rRNA gene, High throughput sequencing, rRNA

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Introduction

Soil is a complex, heterogeneous habitat for a wide variety of organisms, which include bacteria, fungi, protozoan, nematodes and earthworms that play many functional roles in the soil ecosystem. They function as populations or assemblages of similar organisms that interact with each other and their physical environment, thereby contributing to plant nutrition, soil structure, soil fertility, decomposition of organic matter, cycling of nutrients, suppression of soil borne pathogens and removal of toxins [1-3]. The soil microbiota maintains an essential role in biogeochemical cycling, as microbiota are responsible for degradation of organic compounds and govern plant productivity of terrestrial ecosystems. 80- 90 % of the chemical processes in soil are reactions mediated by microbes. [4-5]. One of the most well-known examples is the central role of soil microbes in cycling carbon and nitrogen. Certain species of nitrogen-fixing bacteria mostly from the Actinobacteria phyla are associated with root nodules in order to provide the plants with nitrates [6]. The bacterial community structure may be influenced by a range of environmental parameters, including: pH [7], temperature [8], moisture content [9], nutrient status [10], substrate availability and complexity [11], exposure to the roots of different plant species [12]. Interactions between plants and microorganisms can be classified as pathogenic, saprophytic, and beneficial [13]. Beneficial interactions involve plant growth promoting rhizobacteria (PGPR), generally refers to a group of soil and rhizosphere free-living bacteria colonizing roots in a competitive environment and exerting a beneficial effect on plant growth [14-18]. In the last few decades a large array of bacteria, including species of *Pseudomonas, Azospirillium, Arthrobacteria, Klebsiella, Enterobacter, Alcaligenes, Arthrobacteria, Bacillus* and *Serratia* have been reported to enhance plant growth [19].

The traditional methods of cultivation and isolation of microorganisms involve samples of as little as 0.1% to 1% of soil bacteria [20]. In order to circumvent some of the limitations of cultivation approaches, indirect molecular methods have been developed. Phylogenetic analysis of soil ecosystem has demonstrated that the multitude of discrete prokaryotic species represented in a single sample goes far beyond the numbers and phenotypes of known cultured microorganisms [21-23]. The metagenome sequences can be used to understand the complexity of microbial communities and also how microbes interact within these niches (predict the function of genes present in sequenced genomes). The metagenomic data can be used in further analysis in novel environmental studies as well as in biotechnical and pharmaceutical applications [24]. The 16S rRNA gene sequencing is a widely used alternative for full metagenomic approach to analyze microbial diversity. With 16S rRNA gene sequence data, 95% of genera and 97% of the species are distinguished [25]. The 16S-rRNA consists of regions that are highly conserved between different bacterial and archaeal species, and regions that are highly variable. The former can be used as anchors for the detection of the 16S-rDNA using universal primers in new phylogenetically remote sequences. The latter, the highly variable regions, can provide species-specific signature sequences. The enormous sequence coverage required to analyze less dominant species within a habitat has severely challenged the current computational resources. In response, keeping in mind the importance of Bacterial in the soil ecosystem and the practical power of the metagenomics approach was used to study the diversity and relative abundance of Bacteria present in the soil metagenomics.

MaterialsandMethods

Sample Collection

The soil used in this study was collected from agricultural land of Modasa at (23.47°N 73.3°E), Gujarat, India. The soil was collected by digging 1 cm deep and collected in aseptic plastic bags [26]. Two different types of soil samples (Chandan and Bamboo) were collected in sterile container and transported to the laboratory in cold condition and stored at -20^{0}C for further analysis.

DNA Extraction

DNA extraction was carried out from 0.5gm of each soil samples using CTAB (cetyltrimethyl-ammonium bromide) and SDS(sodium dodecyl sulfate) described by Robert [27] with some modification like filtration and washing of the soil to remove some large molecule followed by phenol: Chloroform classical method. In addition DNA was also isolated with XcelGen Soil gDNA isolation kit (XG2413-01). Finally isolated DNA from both methods was pooled in equimolar concentration to avoid the biasness during DNA isolation process and used for downstream application

PCR based analysis using Ion Tag Primers

PCR amplification of the V6 hyper-variable region of 16S rRNA gene was performed with primers specific for domain prokaryotes [Table-1]. The 5'-ends of the forward primers were fused with the barcoded adaptor plus key sequence (barcode sequences) whereas the reverse primers were fused with the truncated P1-adapter sequence (trP1), respectively. For amplicon library preparation 100 ng of genomic DNA, 0.33 pM of each primer, 0.58 mM of dNTPs, 1X PCR buffer 0.15 mM MgCl2 and 1 U of Taq Polymerase enzyme (Bangalore Genie) were used in 30 µl amplification reaction. The PCR conditions were set as follows: 94°C for 5 min, followed by 25 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 2 min and a final extension step of 72°C for 5 min.

Amplicon product (approx. 165bp) were purified with Ampure XP beads (Agencourt) and the concentration was determined using Agilent High Sensitivity DNA Chip kit on Agilent Bioanalyser 2100 (Agilent Technologies, USA). The libraries were adjusted to a final concentration of 26pM in equimolar concentration and attached to the surface of Ion Sphere particles (ISPs) using Ion One Touch 200 Template v2 sequencing kit (Life Technologies, USA) according to the manufacturer's instructions. Clonally amplified ISP were then enriched by using Ion One Touch ES System resulting in ISPs >95% templated-ISPs. Templated-ISPs were sequenced on Ion 318 semiconductor chip using the Ion Torrent Personal Genome Machine (Life Technologies, USA) for 130 cycles. After sequencing, all raw reads were filtered within the PGM software to remove low quality and polyclonal sequences. All good quality filtered data were exported as *.sff files and subsequently used for bioinformatics analysis.

Table-1 *List of primer targeting regions of 16S rRNA gene used in this study for Amplicon sequencing*

Oligo Name	Oligo Sequence (5' to 3')*	Product size (bp)
V5-Forward	AAACTYAAARRAATTGACGG	165bp
V6-Reverse	CGACRRCCATGCANCACCT	

Metagenomics analysis

All resulting sequencing data sets were uploaded to the Metagenomics - Rapid Annotation using Subsystem Technology (MG-RAST) server (http://metagenomics.anl.gov/) checked for low-quality reads prior to dereplication, annotation and phylogenetic identification. Taxonomic analysis in MG-RAST consisted of comparing the metagenomics sequences with the Non-Redundant Multi-Source Protein Annotation database (M5NR) on the MG-RAST server [28].

Results and Discussion

To investigate the diversity and relative abundance of bacterial species present in the soil. Metagenomic gDNA from soil was sequenced using the Ion Torrent PGM machine. Metagenome sequencing resulted 1,527,454 reads with an average read length of 114 ± 14 bp length in Chandan (MS1) and 1,490,875 reads with an average read length of 112 ± 11 bp length in Bamboo (MS2).

Community structure of Bacteria in soil metagenomics were studied on the basis of M5NR database for 16S rRNA genes at maximum e-value of 1e-5, a minimum identity of 60%. According to M5NR database and sequences features were identified in the given metagenome dataset having putative rRNA regions.

At phylum level, Actinobacteria were predominant in both soil samples, in MS-1(46.84%) and in MS-2(47.06%), followed by Proteobacteria in MS-1(26.80%) and in MS-2(26.91%), Firmicutes in MS-1(23.11%) and MS-2(22.79%) as shown in [Fig-1] and [Fig-2.] This finding is in accordance with studies of the composition of communities in Marine and terrestrial sample and accounted 82% Actinobacteria [29], which is important for, plant health and growth. Proteobacteria communities are an important factor of agriculturally managed systems, as they are responsible for most nutrient transformations in soil and influence the plant diversity and productivity [30]. Helen [31], reported that proteobacteria (82%) was predominant in Soda Lake. In one study 65% of all the bacterial ribosomes originated from Firmicutes [32], which play important role in metabolic activity in soil. The phyla Firmicutes were also more represented in the *P. abies* forest soil [33].

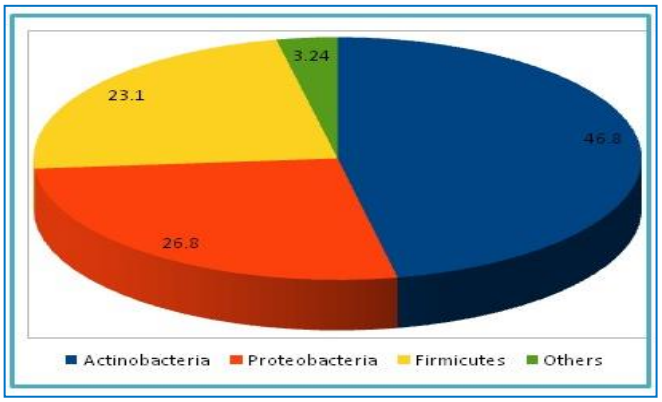

Fig-1 Taxonomic hits distribution of Bacteria at phylum level for Chandan Soil (MS-1)

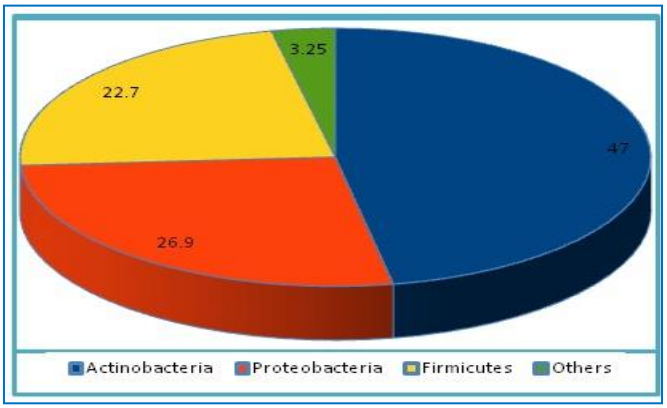

Fig-2 Taxonomic hits distribution of Bacteria at phylum level for Bamboo Soil (MS-2)

Tagged 16 S rRNA Gene sequencing of soil metagenomics revealed the sequences related to 16 genera in soil 1 (MS1) and 18 genera in soil 2 (MS2), there were many other genera below 1% and they may represent different Bacterial species. It was observed that, genera Mycobacterium is dominant in both soil samples, in MS1 (33.18%) and in MS2 (32.97%), similar observation has been reported by Vanessa M. Conn and Christopher M. M. Franco [34] in Swedes Flat soil. The study of bio-solids also revealed high level abundance of Mycobacterium in water sample [35]. Other sequences assign to Lactobacillus (4.5%), Kineococcus (4.06%), Pseudoflavonifractor (3.95%), Vibrio (3.51%), Heliobacterium (3.21%), Brucella (2.85%), Streptomyces (2.76%), Methylococcus (2.48%), Burkholderia (2.38%), 1.8% for Bacillus and Blautia, 1.61% of Rhodococcus and other genera were presented in very low abundance (less than 1%) and accounted for 27.7%(Clostridium, Bartonella, Streptococcus, Staphylococcus) while in sample MS-2 Lactobacillus (4.14%), Kineococcus

(4.29%), Pseudoflavonifractor (3.88%), Vibrio (3.43%), Heliobacterium (3.06%), Brucella (2.78%), Streptomyces (2.87%), Methylococcus (2.32%), Burkholderia (2.45%), 1.74% of Bacillus and 2.03% of Blautia, 1.62% of Rhodococcus and other genera were presented in very low abundance (less than 1%) and accounted for 24.71% (Clostridium, Bartonella, Streptococcus, Staphylococcus) shown in [Table-2]. Kineococcus was also observed in dry Valley mineral soils [36] and in Alaskan soil [37]. However, Heliobacterium genera have been reported by Armando [38] in Brazilian Mangrove Sediments. Streptomyces was previously identified as root colonizers was identify in the study of pant root micobiome [39]. The genus Burkholderia is an important organism of the soil microbial community, which is identified in other study of environmental sample [40]. Methylococcus was observed in sample of environment [41]. Paenibacillus identified in maize rhizosphere [42], Arthrobacter was identified in agricultural soil New Zealand [43] whereas here also in less abundance found, Clostridium was identified in Kenyan soda lake [31], Rhodococcus was found in soil of Antarctica [44] and Pseudomonas was identified in forest soil of albertra, Canada [45].

Table-2 *Abundance of the Bacteria at Genus level present in the Sample MS-1 and MS-2 samples (M5NR)*

Genus	Sample MS-1 (%)	Sample MS-2 (%)
Mycobacterium	33.18	32.97
Lactobacillus	4.5	4.14
Kineococcus	4.06	4.29
Pseudoflavonifractor	3.95	3.88
Vibrio	3.51	3.43
Heliobacterium	3.21	3.06
Brucella	2.85	2.78
Streptomyces	2.76	2.87
Methylococcus	2.48	2.32
Burkholderia	2.38	2.45
Bacillus	1.8	1.74
Blautia	1.8	2.03
Rhodococcus	1.61	1.62
Escherichia	1.55	1.53
Reinekea	1.47	1.39
Coxiella	--	1.1
Bartonella	--	1.02
Synechococcus	1.26	1.3
Others (<1%)	27.62	24.71

Conclusion

The present study reveals the bacterial populations present in both biomaterial with high density. We identified sequences related to 16 genera in soil 1 (MS1) and 18 genera in soil 2 (MS2) which represent different bacterial species. It was observed that, genera Mycobacterium is dominant in both soil samples with high density and it may play important role in dynamics of soil microbial metabolic potential. Other bacterial *Lactobacillus*, *Kineococcus* and *Pseudoflavonifractor* were also appear in both the sample. The present study provides a baseline for understanding the complexity of the soil microbial ecology with special reference to bacterial community. Further, more study is required in large data set to draw a solid conclusion in area of applied agriculture sector.

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Conflict of Interest: None declared

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