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Comparative Evaluation Of Silicate Solubilizing Bacterial Populations In Zea Mays L. Rhizosphere Soils: Integrating Culture Dependent And Culture Independent Methods

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Abstract
Silicon is an essential plant growth mitigator due to its excellent properties in reducing the abiotic and biotic stress of plants. Silicate solubilizing bacteria (SSB) help to convert the insoluble silica present in the earth's crust to soluble form. The current study employed metagenomic analysis to comprehend the microbial profile of the maize rhizosphere. Ten soil samples from the maize rhizosphere were taken from each of the three Indian regions of Joladarasi (Karnataka), Bowrampet (Telangana), and Pappangulum (Tamilnadu). The soil samples from each region were combined into a single sample. Metagenomic sequencing of 16S V3-V4 region showed abundance of Acinetobacter, Lactobacillus, Acetobacter and Bacillus genera. SSB isolates were isolated by using conventional selective medium and were characterized using the 16S rRNA. A total of seven, SSB isolates were isolated, characterized and identified as Bacillus spp. The results of this study demonstrate the potential of Bacillus spp. as silicate solubilizers, which can then be successfully applied as microbial inoculants in agricultural practices.
Keywords: *Bacillus spp., maize, metagenome, silicate solubilizing bacteria*

INTRODUCTION

Zea mays L. is one of the widely cultivated cereal crops globally. Abiotic stress conditions like drought, salinity and alkalinity are showing serious depletion in the crop yields worldwide [1]. Looking into the beneficial aspects of the maize in the food, agriculture and industrial sectors, it is implemented as a model host plant for the research study. Plants in association with the beneficial microbiome can handle the biotic and abiotic stress. Coordination between the plants, soil and microbiome will significantly enhance the plant growth and yield [20,28].

Plants especially are at greatest advantage in getting the necessary elements through the soil. Even though plants have other means of coping with biotic and abiotic stress, research on the metabolism of plants has shown that silicon is the mineral that works best in lowering plant stress [14,25]. The unavailability of silicon in its mono form is a major challenge for the plants, as silica is available in combination with magnesium, calcium, potassium, iron and sodium. This complex form of insoluble silica is only made available in its mono silicic acid either by chemical or biological processes [5]. Bioweathering is a natural process involving microbes which ultimately helps soil in providing elements like silicon, phosphorous as well as potassium for the development of plants [7, 11, 18, 2]. Plants are segregated into three types viz. accumulators, intermediates and excluders based on their silicate accumulation ability. Silica accumulators like maize, paddy and sugarcane deposits high content of silica in their plant parts [17].

From past decades, involvement of microbes in solubilizing minerals in the soil is well documented [8, 27, 32]. Specially, silicon releasing has been evidenced upon degradation of silicates by bacteria [12]. Solubilizing silicates by beneficial microbes has an added advantage to the plants in up taking silica as well as potassium which reduces the need for chemical fertilizers [26]. Bacteria dissolute the natural silicates by many mechanisms such as solubilization using ligands like divalent cations, alkali, inorganic, organic acids and polysaccharides of extracellular origin. Bioweathering of silicates is best done through acidolysis mechanism which is widely accepted. The biotite of silicate mineral solubilization by microbes such as Proteobacteria, Burkholderia, Dyella, Frateuria, Aminobacter, Collimonas, and Janthinobacterium is well documented [31].

For better understanding the molecular level of microorganisms, 16S rRNA is used as a marker especially for soil samples which demarks taxonomical classification as well as phylogenetic classification which excludes the steps of isolation and cultivation of microbes. Identification of bacteria is done using this marker and is still considered as a gold standard [3]. 16S rRNA sequence database has increased

progressively when next generation sequencing has come to use [30]. 16S rRNA has 9 variable regions from V1 to V9 which combinedly contains 1500 bp. Higher ranking taxa can be recognized by the more conservative zones and the rapidly changing areas are used for recognising the genus or species. V3-V4 region in the 16S rRNA has more diversity in its nucleotides and has better identifying ability [34]. α-diversity helps in assessing the taxonomic richness and β-diversity helps in comparing the hypervariable regions [33, 36]. Though various metagenomic data on the microbial profiling of maize rhizosphere were reported, the studies lack comparison between the maize rhizosphere soil samples between different locations in India. Likewise, no information was found regarding the microbial communities that solubilize silicate in the soil samples from the rhizosphere of maize. Keeping the aforementioned in mind, the current research aims to comprehend the role that microbial communities play in the rhizosphere soil of Zea mays L. and assessing the silicate solubilizing bacterial population by using the culture dependent and culture independent methods.

MATERIALS AND METHODS

Collection of soil samples

Samples of the rhizosphere of maize (Zea mays L.) were taken from three states in south India where the crop has been continuously cultivated for the past ten years. Ten samples of soil were taken from Joladarasi, Raichur district, Karnataka (15.7714° N, 76.8015° E), Bowrampet village, Medchal district, Telangana (17.5749° N, 78.3910° E), Pappangulum, Ramanthapuram district, Tamilnadu (9.4097° N, 78.3643° E) from a depth of 10-15 cm near the root zone. Soil sample (100 g) from each location was taken in gamma irradiated containers and were stored at 4°C until processed. Each location's soil samples were combined to create a single sample [22, 25].

Physico-chemical properties of rhizosphere soil

Analysis was done on the physico-chemical characteristics of soil samples from Tamilnadu, Telangana, and Karnataka. Estimates were made for parameters such as electrical conductivity (EC), pH, organic content, nitrogen, phosphorus, and potassium. Using Global DPH507, pH was calculated by combining 1 g of soil with 10 ml of distilled water. The Hanna HI98129 portable conductivity meter was used to measure electrical conductivity. Using the standard techniques, the amounts of nitrogen, phosphorus, and potassium in the soil samples were estimated [21].

DNA extraction and metagenomic sequencing

0.25 g of samples were taken from each rhizosphere soil sample collected and by using DNA extraction kit (Clevergene Biocorp Pvt Ltd, Bangalore), the total DNA was extracted and quantified by Nanodrop (Thermo scientific) at 260/280. For the amplification of V3-V4 region, 25 ng of DNA was taken and amplified by using 341 F (5'CCTACGGGNGGCWGCAG 3') and 785 R (5' GACTACHVGGGTATCTAATCC 3') primers (Clevergene Biocorp Pvt Ltd, Bangalore). PCR was performed by maintaining an initial denaturation of 95°C for 5 min, denaturation at 95°C for 30s, annealing at 55°C for 45s and extension at 72°C for 30s. Final extension was done for 7 min at 72°C. Purification of amplicons was done by 0.9X AMPure XP beads (Beckman Coulter, USA) and eluted with 10µl of 0.1X TE buffer. By using Illumina P7 (AGATCGGAAGAGCACACGTCTGAACTCCAGTCA) and P5 (AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT) barcoding adapters an additional 8 cycles of PCR were run to prepare the sequencing libraries. The obtained PCR products were again purified by AMPure beads and was removed in 15 µl of 0.1X TE buffer. Concentration of prepared libraries was determined by Qubit™ 3 Fluorometer (Life Technologies, USA) using the dsDNA broad range assay kit (ThermoFisher Scientific, USA). Dye and the buffer present in the kit were diluted in 1:200 ratio to which 1 µl of library was mixed and incubated for 2 min at room temperature. Readings were recorded by using Qubit™ 3 Fluorometer [15].

Quality check of the amplicon

Unprocessed DNA sequence information was obtained from the Illumina MiSeq and the quality check was performed by using FastQC software. The collected data was examined for the distribution of base call quality and percentage of bases which are above Q30 and Q20 were selected. Similarly, contamination of sequence adapters and GC% were assessed. The soil samples with Q20 above 95% were selected and further studied [35].

Data Analysis

From the 5' end, reads (~20 bp) were trimmed to remove the degenerate primers. Low quality bases and adapter sequences were eliminated with the aid of Trimgalore. Contigs were formed by importing good

quality sequence to Mothur for alignment, errors were screened and the contigs in between 300 – 532 bp were selected. Uncertain base calls were eliminated and the identical or duplicate sequences were merged. Alignment of contigs were done based on the known database of 16S rRNA. Gaps and overhang areas from the contigs that might have developed as a result of mistakes made during the PCR amplification were removed. The screened contigs were segregated into taxonomical outlines using GREENGENES v.13.8-99 database. Contigs were clustered into individual operational taxonomic units (OTU) and abundance of the microbial population was estimated [9].

Screening of silicate solubilizing bacteria by using culture dependent method

Samples of the rhizosphere were diluted serially and inoculated on the selective media with composition (g/L): 10 glucose, 1 yeast extract, 2.5 magnesium trisilicate with 20 g agar by standard plate count method. Inoculated medium plates were incubated for 96 h at 30°C. Silicate solubilizing bacterial isolates showing silicate solubilization zones were screened and purified. Efficient isolates obtained were subcultured on Soyabean casein digest agar slants and stored at 4°C [13, 17].

Molecular characterization of silicate solubilizing isolates

Potent silicate solubilizing bacterial isolates showing high zone of solubilisation were characterized by using the 16S rRNA sequencing technique. Silicate solubilizing bacterial cultures were inoculated into 250 ml of Luria Bertani broth. The culture flasks were kept on the orbital shaker (REMI CIS 24) for 12 h. By using Quick-DNA midiprep kit (Zymo research - D6105), After extracting the DNA, amplification was done using 518 F- CCAGCAGCCGCGGTAATACG and 800 R – TACCAGGGTATCTAATCC primers. DNA sequencing was outsourced (Bioserve Biotechnologies, Hyderabad) and the chimeric sequences in raw DNA sequence was checked and removed by using Chromaspro 2.0 software. DNA sequences obtained were queried in the NCBI BLAST and based on the similarity score was used to identify the bacterial isolates. DNA sequences obtained were deposited in the NCBI Genbank. The DNA sequences were aligned using ClustalW for phylogenetic analysis, and MEGA X was used to create a neighbor joining tree using 500 bootstrap replications [6, 29].

RESULTS

Rhizosphere soil's chemical and physical characteristics

Samples of the rhizosphere of maize were taken from three different states of India where maize was under continues cultivation. Samples of rhizosphere soil were taken from Joladarasi of Karnataka, Bowrampet of Telangana and Pappangulum of Tamilnadu which were labelled as SSKA01, SSTG02 and SSTN03. Physico-chemical properties of the rhizosphere samples were assessed. The pH parameter for SSTN03 and SSKA01 were in between the standard values but SSTG02 showed more alkaline nature. Electrical conductivity and organic content for all the three soils were below the standard range. Average nitrogen for SSTG02 and SSTN03 are lower than the standard range and SSKA01 lies in between the range. Average phosphorous for all the three soils are below the standard range. Average potassium for SSKA01 and SSTN03 are in between the standard range and SSTG02 lies below the range (Table 1).

Table 1. Physico-chemical parameters of the soil samples

Parameters	Standard values	Karnataka (SSKA01)	Telangana (SSTG02)	Tamilnadu (SSTN03)
pH	6.5-7.5	7.4	8.15	6.8
EC (dS/m)	1.5-5.7	0.21	0.26	0.32
Organic content (%)	0.5-0.75	0.56	0.41	0.51
Average N (Kg/Ha)	280-560	302	167	248
Average P (Kg/Ha)	10.0 -25	4.1	4.36	5.9
Average K (Kg/Ha)	110-260	167	131	184

DNA extraction and metagenomic sequencing

After extracting DNA from the rhizosphere soil samples, 16S rRNA's V3–V4 region was amplified. Quantification of the sequence libraries was done by using Qubit™ 3 Flourometer which showed 11.2 ng/µl, 13.5 ng/µl and 9.8 ng/µl for Karnataka rhizosphere soil (SSKA01), Telangana rhizosphere soil (SSTG02) and Tamilnadu rhizosphere soil (SSTN03) which passes as per the standards.

Quality check of the DNA sequences

After being examined for quality using the FastQC program, the acquired raw DNA sequences demonstrated high base quality. Examining the rhizosphere soil samples for quality showed more than 95% of Q20 that is 99.470, 99.225, and 99.320 for the Karnataka rhizosphere soil (SSKA01), Telangana rhizosphere soil (SSTG02) and Tamilnadu rhizosphere soil (SSTN03) respectively. The DNA sequencing from the three soil samples (SSKA01, SSTG02, SSTN03) using Illumina MiSeq resulted in a sequence reads of 163748, read length of 301 bp and 53.5% GC content for Karnataka rhizosphere soil (SSKA01). Number of reads are 132814, read length of 301 bp and GC content of 53.5% was obtained for Telangana rhizosphere soil (SSTG02). Similarly, for Tamilnadu rhizosphere soil (SSTN03) number of reads are 127726, read length of 301 bp and 54.5% GC content respectively (Table 2).

Table 2. Raw DNA sequence data depicting the quality of the DNA sequence

Sample name	Sample Code	No. of Reads	GC %	Read length	%Q20
Karnataka	SSKA01	163748	53.5	301	99.470
Telangana	SSTG02	132814	53.5	301	99.225
Tamilnadu	SSTN03	127726	54.5	301	99.320

Contig length distribution and PCoA analysis

The low-quality bases, primers and sequences of the adapters were cut using the Trimgalore software and the good quality bases were uploaded to the Mothur for further construction of contigs. The contigs in between 300-532 bp were screened and selected.

The PCoA (principal coordinates analysis) is used to analyse the similarities of data and variation among the samples. Similar groups are represented by the same colour. Variations in the sample coordinates are shown in percentage which reveals the percentage of information retrieved from the obtained data. The similarity of the microbial communities is revealed by the distance between the soil samples. The microbial communities of the rhizosphere soil samples from Telangana (SSTG02), Tamilnadu (SSTN03), and Karnataka (SSKA01) were found to be unique from one another using PCoA. Tamilnadu rhizosphere soil (SSTN03) is ($p < 0.06$), Karnataka rhizosphere soil (SSKA01) is ($p > 0.2$), and Telangana rhizosphere soil (SSTG02) is the intermediate of the two samples (Fig. 1).

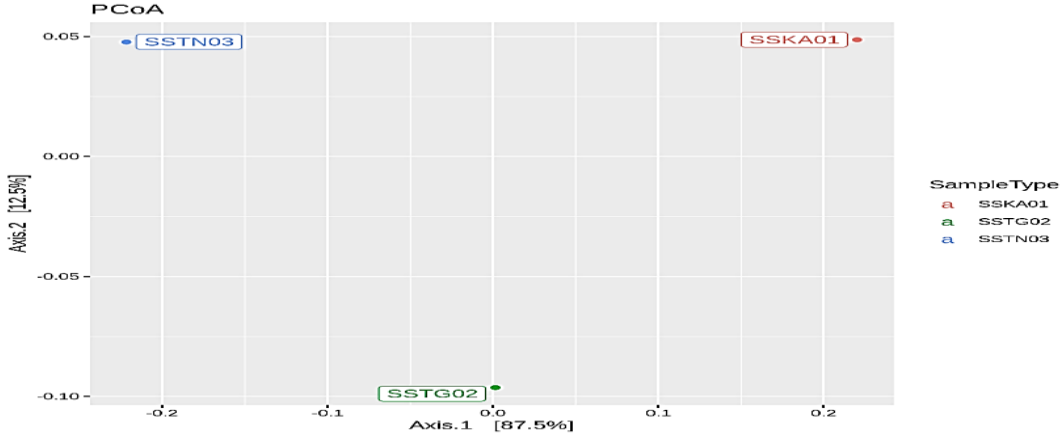

Fig. 1. PCoA plot to check the similarity between the rhizosphere soil samples based on their taxonomical distribution

Microbial abundance in rhizosphere soil of Zea mays L.

The microbial abundance for the three rhizosphere soil samples of maize were analysed for all the taxonomic ranks and were represented in stacked bar graphs and heat maps. The phylum abundance in the soil samples showed richness of Firmicutes followed by the Proteobacteria. Karnataka rhizosphere soil (SSKA01) has 43.17% of Firmicutes and 39.59% of Proteobacteria which is highest abundance percentage when compared to the other two samples. Abundance of Firmicutes was 40% and 36.0% of Proteobacteria was recoded in Telangana rhizosphere soil (SSTG02) whereas a slight variation in the microbial abundance was observed in Tamilnadu rhizosphere soil (SSTN03) which has 33% of Proteobacteria and 27% of Firmicutes. When all the three samples were compared, it showed high microbial abundance of Firmicutes and Proteobacteria (Fig. 2). Excluding the Firmicutes and Proteobacteria, phyla like Bacteriodes, Planctomycetes, Actinobacteria, Cyanobacteria, Chloroflexi, Acidobacteria and Verrucomicrobia were also notified.

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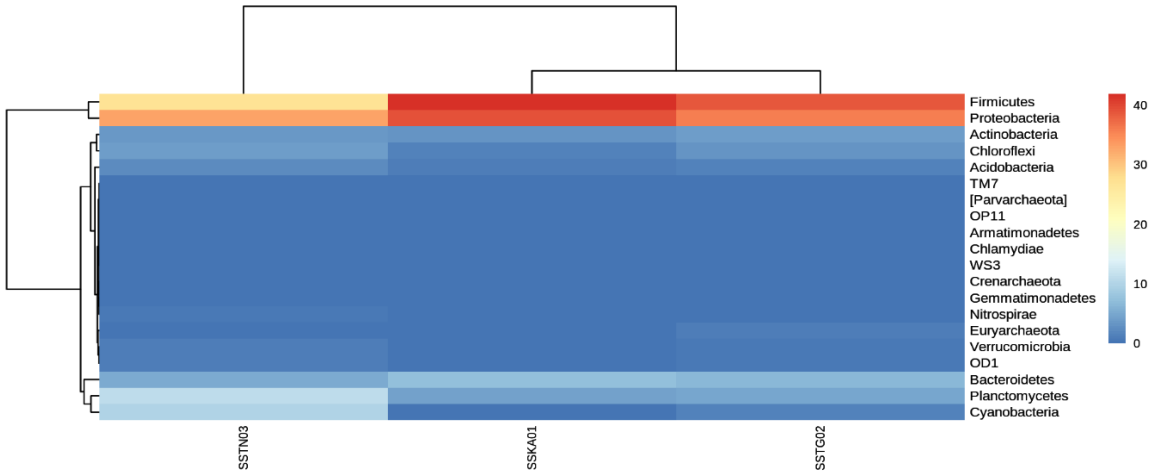


Fig. 2. Heat map showing the abundance of phylum in the three rhizosphere soils of Zea mays L. collected from Karnataka, Telangana and Tamilnadu

Microbial abundance at the genus level was assessed for the tested soil samples. In Karnataka rhizosphere soil (SSKA01), high genus abundance was recorded for Acinetobacter 35% followed by Lactobacillus 19.25%, Acetobacter 16% and Bacillus 9.09% whereas Telangana rhizosphere soil (SSTG02) showed Acinetobacter 30.5% followed by Lactobacillus 19.0%, Bacillus 15.2% and Acetobacter 11.38%. Genus abundance in Tamilnadu rhizosphere soil (SSTN03) was Acinetobacter 28.8% followed by Lactobacillus 10.7%, Bacillus 13.1% and Acetobacter 6.34%. Genus abundance of Lactobacillus in both Karnataka rhizosphere soil (SSKA01) and Telangana rhizosphere soil (SSTG02) were almost similar. When compared to the Telangana rhizosphere soil (SSTG02) and Tamilnadu rhizosphere soil (SSTN03), Karnataka rhizosphere soil (SSKA01) recorded less abundance of Bacillus. These three rhizosphere soil samples also include the genera Dysgonomonas, Aeromonas, Elizabethikingia, Enterococcus, Gemmata and Macellibacteriodes (Fig. 3). The genus distribution is well represented in the Krona pie chart which shows the depiction of microbial abundance and hierarchy simultaneously.

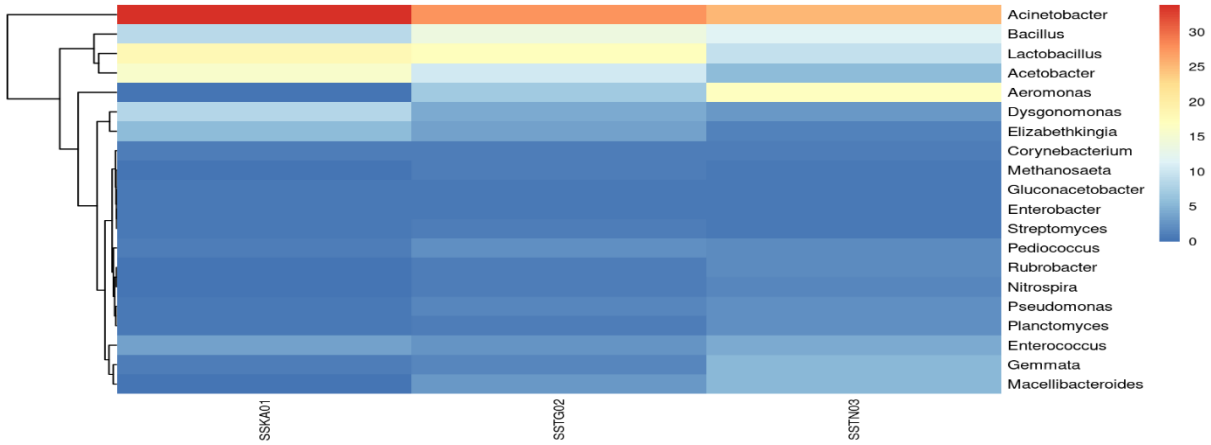


Fig. 3. Heat map showing the abundance of genus in the three rhizosphere soils of Zea mays L. collected from Karnataka, Telangana and Tamilnadu

Alpha diversity

Alpha diversity is the study of diversity of individual sample which also studies the diversity abundance of species in a given microbial population. The indices Chao and Abundance based coverage estimator (ACE) were used to estimate the microbial community richness whereas Shannon and Simpson were used to study the microbial community diversity calculation. According to the alpha diversity index Chao1, the Telangana rhizosphere soil (SSTG02), or 1129.67, has a high concentration of unique OTUs (richness), followed by the Karnataka rhizosphere soil (SSKA01, 955.01) and the Tamilnadu rhizosphere soil (SSTN03, 861.59). The above Chao index results indicate the abundance and richness of the microbial communities in the Telangana rhizosphere soil SSTG02 sample. ACE index results showed 1072.80 in Telangana rhizosphere soil (SSTG02), 936.40 in Tamilnadu rhizosphere soil (SSTN03) and 864.6 in Karnataka rhizosphere soil (SSKA01). The results of ACE and Chao1 correlates with each other indicating the abundance and richness of microbial communities. When the sampling groups were statistically analyzed, the Shannon diversity index revealed a distinct pattern, showing unique OTUs in the

rhizosphere soils of Tamilnadu (SSTN03 (4.38), Telangana (SSTG02 (4.33), and Karnataka (SSKA01 (3.91). Interestingly, Tamilnadu rhizosphere soil SSTN03 and Telangana rhizosphere soil SSTG02 samples have very near values which shows the uniformity and richness in both the samples (Table 3). Results of Simpson index also correlates with the Shannon index results. The microbial abundance between the samples and the groups were represented in (Fig. 4).

Table 3. Alpha diversity measurements of three rhizosphere soils of Zea mays L.

Name of the sample	Sample	Observed	Chao1	ACE	Shannon	Simpson
Karnataka	SSKA01	656	861.59	864.60	3.91	0.95
Telangana	SSTG02	795	1129.67	1072.80	4.33	0.96
Tamilnadu	SSTN03	758	955.01	936.40	4.38	0.95

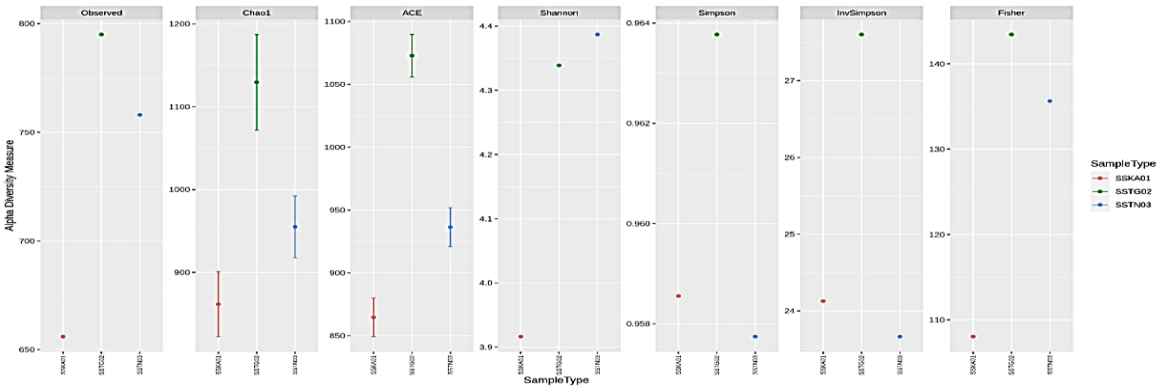
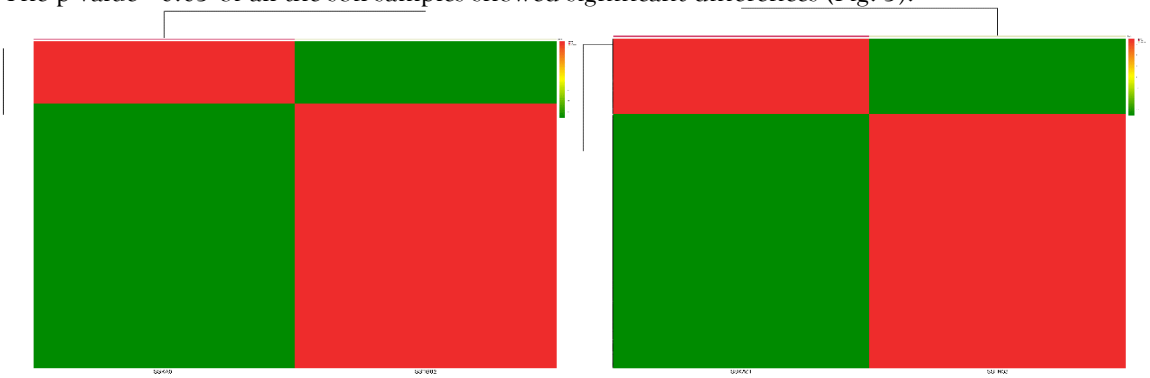


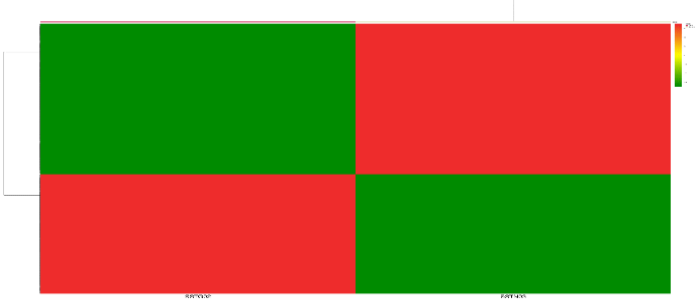
Fig. 4. Alpha diversity of rhizosphere soil samples of Karnataka rhizosphere soil (SSKA01), Telangana rhizosphere soil (SSTG02) and Tamilnadu rhizosphere soil (SSTN03)

Beta Diversity Analysis

Microbial diversity between the samples is best studied by using the beta diversity. Fisher's exact test was used to determine whether there was a statistically significant difference in OTU abundance between the samples using the STAMP method. Comparison between Karnataka rhizosphere soil (SSKA01) vs Telangana rhizosphere soil (SSTG02) showed significant OTUs with 139 (corrected p-value <0.05) and Karnataka rhizosphere soil (SSKA01) vs Tamilnadu rhizosphere soil (SSTN03) showed 206. Similarly, Telangana rhizosphere soil (SSTG02) vs Tamilnadu rhizosphere soil (SSTN03) showed the p value of 107. The p value <0.05 of all the soil samples showed significant differences (Fig. 5).



SSKA01 Vs SSTG02 **SSKA01 Vs SSTN03**



SSTG02 Vs SSTN03

Fig. 5. Beta diversity of the rhizosphere soils of Zea mays L. from Karnataka (SSKA01), Telangana (SSTG02) and Tamilnadu (SSTN03). The correlation coefficients range is -1 to 1.

Silicate solubilizing bacteria from isolated three rhizosphere soil samples

Metagenomic results revealed the microbial community profile in the sampling locations of Karnataka rhizosphere soil (SSKA01), Telangana rhizosphere soil (SSTG02) and Tamilnadu rhizosphere soil (SSTN03) of India. With an aim to isolate the silicate solubilizing bacteria, the rhizosphere samples were serially diluted and inoculated on the conventional selective media. Total seven potent silicate solubilizing bacteria were isolated and by using 16S rRNA the isolates were identified (Fig. 6).

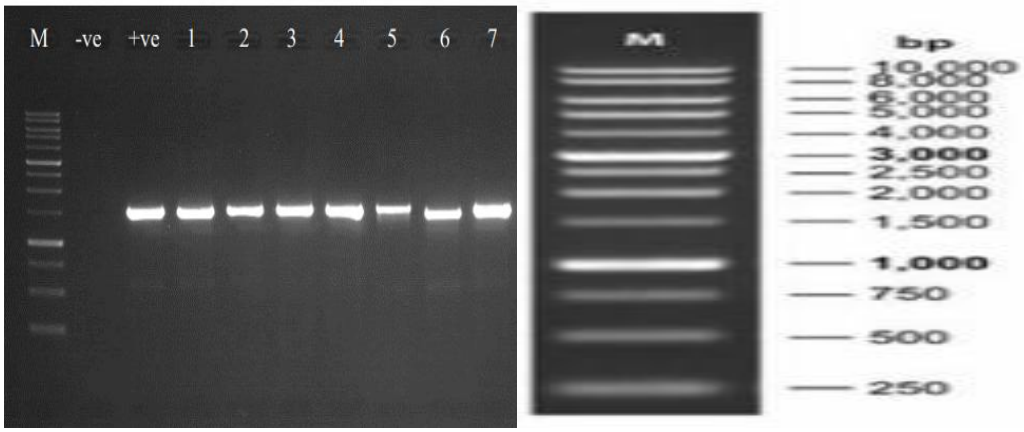


Fig. 6. Agarose gel electrophoresis (2%) 16S rRNA gene amplified from seven silicate solubilizing bacteria. Lanes 1&2 Karnataka isolates, Lanes 3&4 Telangana isolates and 5-7 Tamilnadu isolates. M represents ladder DNA of 10,000 base pairs

DNA sequencing was done and the sequences obtained were queried in the NCBI BLAST for potent silicate solubilizers matching. The acquired gene sequences were added to the NCBI Genbank and given accession numbers. Out of the seven SSBs, Bacillus vallismortis SK070 (MT093348) and Bacillus tequilensis SKSSB09 (MW405905) were isolated from Karnataka rhizosphere soil (SSKA01) sample. From Telangana rhizosphere soil (SSTG02) sample, Bacillus megaterium SKSSB18 (MW405907) and Bacillus subtilis SKSSB22 (MW405911) were isolated. Enterobacter hormachei SSB041(MW664035), Bacillus paramycoides SSB029 (MW664369) and Bacillus velezensis SSB036 (MW665109) were isolated from the Tamilnadu rhizosphere soil (SSTN03) sample. Neighbour joining tree was constructed by using the MEGA X with 500 bootstrap replications (Fig. 7).

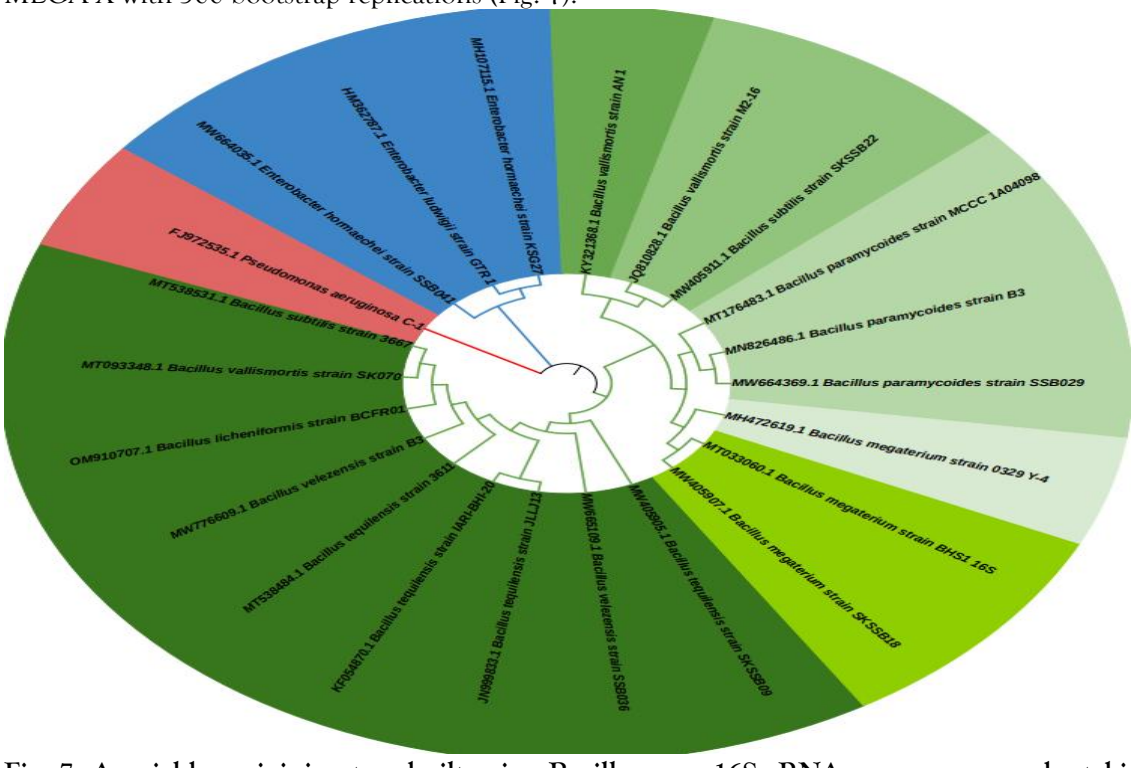


Fig. 7. A neighbour joining tree built using Bacillus spp. 16S rRNA gene sequences by taking 500 bootstrap replications.

DISCUSSION

Rhizosphere microbiome performs multiple beneficial activities in the agricultural and ecological perspective. Beneficial plant microbes are proven to be showing useful effects on the plants by promoting growth in various developmental stages of maize. Beneficial microbes in plants are essential for preserving the soil's health and the biogeochemical cycle of nutrients [4]. In the present study, comparison of rhizosphere microbiome of maize from three several states in South India were examined and silicate solubilizing bacteria were isolated by using culture dependent conventional method. The physicochemical characteristics (pH, EC, organic content, nitrogen, phosphorous, and potassium) of the rhizosphere soil samples were examined before moving on with the metagenomic sequencing. All the three samples of rhizosphere soil recorded low electrical conductivity, organic content, phosphorous and potassium. The rhizosphere soil samples of Karnataka (SSKS01) and Tamilnadu (SSTN03) recorded medium nitrogen content whereas Telangana (SSTG02) recorded less nitrogen content. Telangana soil sample recorded low values for every physicochemical properties tested. Comparison of the physicochemical properties of all the three rhizosphere soil samples showed that the soils collected from the three areas may not be a good habitat for the beneficial soil microbial population as there is low organic content, nitrogen, phosphorous and potassium contents. In this scenario, only the abundant microbial population which habituated from a long time in the rhizosphere can be expected in the current research. Previous research reports also recorded similar physicochemical properties for the Karnataka, Tamilnadu and Telangana rhizosphere soils which showed low to medium values for nitrogen, phosphorus, potassium, electrical conductivity and organic content. Low levels of organic content, nitrogen, phosphorous and potassium also reveals the negative effect of usage of synthetic fertilizers [10, 21]. Similar physicochemical parameters tested for Kallar Syedan in Pakistan recorded better values i.e., EC (0.72-0.79 dsm^{-1}), pH (7.67-7.77), organic content (0.71-1.21), phosphorous (6.9-7.1 mg/kg) and potassium (100-120 mg/kg) in both bulk soil and rhizosphere soil [16]. Physicochemical parameters tested for bulk soil and rhizosphere soil of Lichtenburg and Randfontein, South Africa also recorded low results in Lichtenburg for organic content - 0.6-0.61, pH – 5.62-5.87 and phosphorous – 50.98 – 65.86 mg/kg when compared to Randfortein (organic content - 0.67-0.87, pH – 6.73-6.76 and phosphorous – 206 – 257.14 mg/kg). Whereas the same soil samples showed high potassium (16.24-16.29 mg/kg) and nitrogen (240 – 243 mg/kg) in Lichtenburg when compared to Randfontein (potassium – 7.38 – 8.52 mg/kg, nitrogen – 148 - 167 mg/kg) [4]. In general, organic carbon, nitrogen, phosphorous and potash play a crucial part in the development of the plants and beneficial microbes. Depletion of these elements will cause the soil to deteriorate, which will then have an impact on plant growth and the populations of microorganisms living in the soil. Similarly, pH of the soil plays a key role in modulating the physiology of plants and rhizosphere microbial communities [20].

The Illumina Hiseq platform was utilized to process the soil samples from the rhizosphere in order to estimate the relative abundance and diversity of microorganisms by metagenomic sequencing. The V3-V4 region sequence revealed that the number of high-quality reads with a read length average of 301 bp and Q20 >99% was 163748 for the Karnataka soil sample (SSKA01), 132814 for the Telangana soil sample (SSTG02), and 127726 for the Tamilnadu soil sample (SSTN03). The PCoA analysis showed the similarities between the data of three rhizosphere soil samples in which Telangana rhizosphere sample (SSTG02) is the intermediate between the Karnataka rhizosphere sample (SSKA01) and Tamilnadu rhizosphere sample (SSTN03). Phylum abundance showed high percentage of Firmicutes and Proteobacteria whereas Bacteriodes, Planctomycetes, Cyanobacteria, Actinobacteria, Verrucomicrobia, Acidobacteria and Chloroflexi in low abundance. The results obtained were in agreement with the previous research reports. Maize rhizosphere from the Argentina showed high phylum abundance of Proteobacteria and Firmicutes which was studied in both culture dependent and culture independent methods [24]. Where as in the Litchtenburg and Randfontein, South Africa maize rhizosphere soils, high phylum abundance of Proteobacteria, Actinobacteria, Acidobacteria, Gemmatomonadetes, Bacteriodetes and Verrucomicrobia were noticed [4]. This report is also in accordance with the maize rhizosphere of Kallar Syedan, Pakistan which showed high phylum abundance of Proteobacteria, Actinobacteria, Acidobacteria, Firmicutes, Chloroflexi, Bacteriodetes, Verrucomicrobia, Thermomicrobia, Nitrospirae and Gemmatomonadetes [24, 33]. From the previous research reports, Proteobacteria phylum showed high abundance irrespective of the geographical locations where as abundance of Firmicutes is inconsistent.

Genus abundance of the three maize rhizosphere soils of south India showed high genus abundance of Acinetobacter, Lactobacillus, Acetobacter and Bacillus. Whereas Dysgomonas, Aeromonas, Elizabethikingia, Enterococcus, Gemmata and Macellibacteriodes were recorded in low abundance. This was contradicted by the results obtained for maize rhizosphere of Kallar Syedan, Pakistan, in which Bacillus, Sphingomonas, Microvirga, Bryobacter, Rubrobacter, Nocardioides, Marmaricola, Solirubrobacter, Devosia and Gaiella were dominant genera [24, 33]. Similarly, in the maize rhizosphere of Litchtenburg and Randfontein, South Africa, high abundance of Gemmatimonas, Bacillus, Conexibacter, Streptomyces, Mesorhizobium, Pseudomonas, Gemmata, Burkholderia, and Micromonospora were significantly recorded [4]. In a global scenario, maize rhizosphere contains high abundance of Bacillus, Paenibacillus and Staphylococcus genera which comes under the Firmicutes phylum. Similarly, Acinetobacter, Enterobacter, Achromobacter, Azotobacter and Rhizobium as high abundant genera belonging to Proteobacteria phylum [19]. The genera abundance in the present research is in coordination with the other reports and on a common scale Bacillus genus was recorded in all the research findings. The slight variations in the microbial communities will be influenced by the maize root exudates, soil physicochemical properties and the accessibility of nutrients [20]. The Telangana rhizosphere soil (SSTG02) had unique OTU richness and high microbial diversity in the Tamilnadu rhizosphere soil (SSTN03), according to the calculation of alpha diversity based on the Chao, ACE, Shannon, and Simpson indices. Shannon index more than 1 indicates the high microbial diversity in the soil sample [23]. Beta diversity studies between the three rhizosphere soil samples showed positive correlation between the microbial communities. The fisher's extract test with STAMP was used in the previous research for studying the correlation between the samples [24].

With a perspective to study the silicate solubilizing population of microbes in the rhizosphere of maize, samples were screened on the conventional selective media. 16 S rRNA sequencing was used to characterize the silicate solubilizing isolates and obtained DNA sequence was deposited in Genbank. Out of the 7 SSB strains obtained, 2 isolates were from Karnataka rhizosphere (SSKA01), 2 isolates were from Telangana rhizosphere (SSTG02) and 3 isolates were from Tamilnadu rhizosphere soil (SSTN03). Most of the isolated silicate solubilizing strains belongs to the Bacillus genera and only one strain belongs to the Enterococcus genera. Out of the 4 major genera identified in the metagenomic analysis, Bacillus genus occupies a key portion as it has a greater number of silicate solubilizing bacterial isolates. Though there are other genera abundantly available in the soils, there role may be different which may include other plant growth promoting properties.

Bacillus spp. possess a remarkable ability to form endospores which makes them to withstand in harsh environmental conditions. Bacillus spp. were good silicate solubilizing bacteria as they secrete high quantities of organic acids which dissolute the insoluble silicates [27]. Their presence in the 3 sampling sites shows how stable they are to the biotic and abiotic stress and helps maize crop in supplying silica in soluble plant available form. The culture dependent and culture independent studies on the maize rhizosphere of Argentina showed the abundance of Bacillus, Pseudomonas, Enterobacter, Pantoea and Klebsiella [24]. The culture dependent results obtained in the current research is in coordination with the previous research reports.

Though there are maize rhizosphere metagenomic analysis reports recorded previously, the current work is the first report on the microbial communities of maize rhizosphere of south Indian states (Karnataka, Telangana and Tamilnadu). As on date, no research reports were noted on the identification of silicate solubilizing bacteria in the maize rhizosphere. The present research work is the first report notifying the silicate solubilizing bacterial population in the maize rhizosphere which was studied by using culture dependent and culture independent methods.

CONCLUSION

Examining the microbial communities in the three rhizosphere soil samples from south Indian states was the main goal of the current study. Metagenomic study revealed the abundance of microbial population and 4 major genera viz. Acinetobacter, Lactobacillus, Acetobacter and Bacillus were notified. The isolation of silicate solubilizing bacteria by conventional culture dependent methods, revealed the abundance of Bacillus spp. which shows the potentiality of Bacillus genus as silicate solubilizers. This study shows the impact of silicate solubilizing Bacillus spp. on maize growth and yield.

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Conflict of Interest

Authors have no conflict of interest

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