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RESEARCH ARTICLE

Bacterial community structure analysis of soil treated with *Parthenium hysterophorus* L. derived green medium

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ABSTRACT

The present study encompasses the analysis of bacterial community structure of soil in the presence of *Parthenium hysterophorus* derived green medium. The 16S microbiome profiling of the soil revealed that it consists of members from 15 bacterial phyla with the most prominent being Proteobacteria. The other predominant phyla were Plantomycetes, Actinobacteria, Acidobacteria, Bacteroidetes, Chloroflexi and Firmicutes. The maximum proportion of the bacterial community remained unclassified at genus and species level. Among the classified population the maximum number of bacteria belonged to *Flavisolibacter* followed by *Kaistobacter*, *Bacillus*, *Optitutus*, *Balneimonas*, *Steroidobacter*, *Rhodoplanes* and *Gemmata*.

Introduction

Soil habitats probably contain the greatest microbial diversity of all the environments on earth (1, 2). This is because of the availability of various macro and micro elements, water and organic matter which makes it an ideal habitat for microbial growth. These microorganisms are essential part of an ecosystem and play crucial role in various environmental cycles such as Nitrogen cycle, Carbon cycle etc (3). In addition to this, they have vast biotechnological importance (4) and their utility in agriculture sector is well recognized for centuries (5). The soil microorganisms have potential to increase crop yields and quality by their beneficial traits like phosphate solubilization, potassium solubilization, production of plant growth hormones etc (6, 7). The abundance and distribution of these microorganisms in soil depends on various factors such as soil texture and structure, pH, nutritional composition, humidity etc (8).

In the current scenario of agricultural system, use of organic farming and leaf based compost is greatly promoted due to the increasing cost of chemical fertilizers and some of their negative environmental impacts (9). The organic compost is highly effective in terms of increasing total nitrogen (N) concentration, organic matter, microbial biomass as well as enzyme activities in the soil (10). Better yield of crops have been obtained by incorporating leaf compost or other forms of plant residues in the soil (11). It has also been revealed that such green manuring approaches increase soil organic matter and microbial biomass for long-term (1, 12, 13). Since soil fertility conferred by incorporation of organic material involves microbial action, it is of utmost importance to determine the bacterial community structure of soil in the presence of green manures and composts. The classical method for determining microbial diversity of an environment requires culturing and therefore, has many limitations (14). In place of this, the culture independent approach of metagenomics is often being used to acquire comprehensive details of the whole microbial community structure of an environment (15, 16). We had earlier documented the efficiency of a culture medium developed from *Parthenium hysterophorus* leaves for isolation of plant growth promoting bacteria (17). The present work is an extension to that and describes the 16S microbiome profiling of soil in the presence of a green medium derived from *P. hysterophorus*.

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Materials and Methods

Preparation of Parthenium based green medium

The green medium in the form of a fine powder was prepared from the leaves of *Parthenium hysterophorus* L. growing in the Chauras campus, HNB Garhwal University, Srinagar Garhwal, India (17). The taxonomic identification of plant was confirmed at the Botanical Survey of India, Dehradun with herbarium no. ACC 505.

Soil sample collection and soil amendment

The soil sample was collected from a depth of 0 to10 cm below earth's surface from the garden of Department of Zoology & Biotechnology (78'48'10" E longitude and 30º 13'36" N latitude) located in Chauras campus of HNB Garhwal University Srinagar, Uttarakhand, India at the bank of river Alaknanda. The depth selection was based on the possibility of trapping the largest population of soil bacteria both aerobic and anaerobic. The soil of the campus is already known to be of sandy loam texture with pH near neutrality (18). Agricultural practices are undertaken by nearby villagers in the fields with similar soil characteristics and therefore, it was selected for the present work. Two kg of soil was taken to the laboratory, where it was mixed all at once with 2 gm powder of green medium prepared from *Parthenium hysterophorus* (17). The mixture was filled in a polybag and the same was kept under open environmental conditions. After 15 days, 30 gm of soil sample was collected from the polybag at a depth of 5–6 cm with the help of a sterile spatula. The same was kept in a pre-sterilized plastic bag and sent to Eurofins Genomics India pvt. Ltd., Bengaluru, India for carrying out 16S microbiome profiling in the following manner.

DNA extraction, amplification and library preparation

The metagenomic DNA was extracted from the soil sample with the help of a DNA isolation kit (Nucleospin Soil). For determining the purity, 1 µl of DNA sample was loaded in NanoDrop for determining A260/280 ratio.

Library preparation

The amplicon libraries were prepared using Nextera XT Index Kit (Illumina inc.). Primers with the sequences 16S rRNA F GCCTACGGGNGGCWGCAG and 16S rRNA R ACTACHVGGGTATCTAATCC were used to amplify 16S rDNA gene targeting the bacterial V3-V4 region. Electrophoresis was carried out with 3 µl of PCR product on 1.2 % of agarose gel at 120v for approximately one hour to check the presence of correctly amplified targeted region. The amplicons with the Illumina adaptors were then amplified through i5 and i7 primers that added multiplexing index sequences as well as common adapters required for cluster generation (P5 and P7). The purification of amplicon libraries was done by AMPureXP beads and the same was quantified using Qubit fluorometer.

Sequencing and bioinformatics analysis

The amplified libraries were analyzed on 4200 tape station system (Agilent technology) using D1000 screen tape for quality and quantity check. After obtaining the mean peak size from Tape Station profile, the libraries were loaded onto MiSeq at appropriate concentration (10–20pM) for cluster generation and sequencing. The bioinformatics analysis was performed using QIIME comprehensive software. All the sequences from the sample were cluster into Operational Taxonomic Units (OTUs) based on their sequence similarity at 97%. The library was sequenced on MiSeq using 2 X 300 bp.

Results

Metagenomic DNA extraction, amplification and library preparation

The metagenomic DNA was successfully extracted and its concentration was determined as 122 ng/µl. The A260/280 ratio for it was calculated as 1.87 declaring this to be in a highly pure form to be utilized for the further analysis. The V3–V4 regions of 16S rDNA was amplified (Fig. 1) and amplicon library was successfully prepared with mean of the library

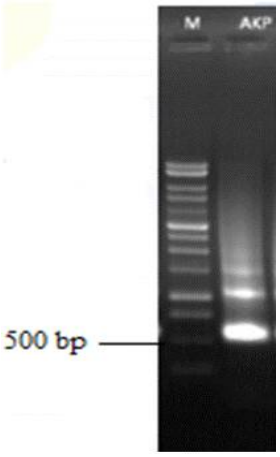
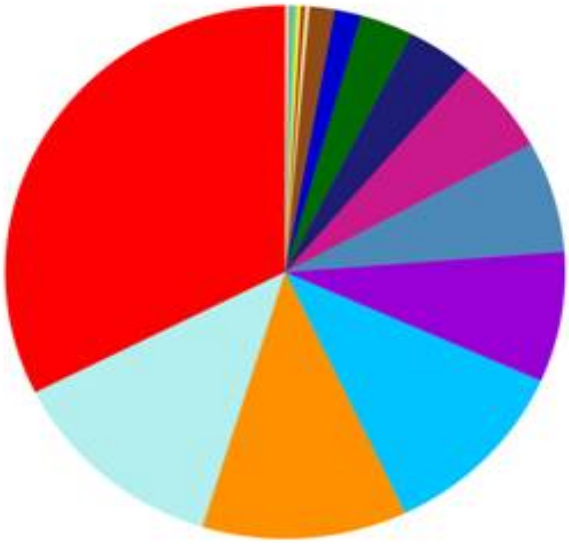


Fig. 1. Amplified V3-V4 region of 16S rDNA on agarose (1.2%) gel.

fragment size distribution being 596 bp. In the sequencing process a total of 249135 reads resulted in 116 Mbp of data output.

Bacterial community structure analysis

The bioinformatics analysis revealed that the bacterial community structure of the soil treated with the *Parthenium* derived green medium comprised 15 bacterial phyla including five unclassified bacterial group (Fig. 2). Predominance of Proteobacteria was noticed in the soil sample comprising 32.45% of the total population. The abundance of other phyla was in the following order as Planctomycetes (12.77%), Actinobacteria (11.84%), Acidobacteria (11.26%), Bacteroidetes (7.9%), Chloroflexi (6.75%), Firmicutes (5.77%), Verrucomicrobia (3.82%), Gemmatimonadetes (3.06%), Nitrospirae (1.53%), Cyanobacteria (1.46%), Euryarchaeota (0.25%), Armatimonadetes (0.24%), Chlorobi (0.22%) and Fibrobacters (0.11%). The total percentage of unclassified groups was upto 0.57%. At class level, the



Legends	Taxonomy	Abundance
	k__Bacteria;p__Proteobacteria	32.45%
	k__Bacteria;p__Planctomycetes	12.77%
	k__Bacteria;p__Actinobacteria	11.84%
	k__Bacteria;p__Acidobacteria	11.26%
	k__Bacteria;p__Bacteroidetes	7.9%
	k__Bacteria;p__Chloroflexi	6.75%
	k__Bacteria;p__Firmicutes	5.77%
	k__Bacteria;p__Verrucomicrobia	3.82%
	k__Bacteria;p__Gemmatimonadetes	3.06%
	k__Bacteria;p__Nitrospirae	1.53%
	k__Bacteria;p__Cyanobacteria	1.46%
	k__Archaea;p__Euryarchaeota	0.25%
	k__Bacteria;p__Armatimonadetes	0.24%
	k__Bacteria;p__Chlorobi	0.22%
	k__Bacteria;p__TM7	0.14%
	k__Bacteria;p__WS3	0.13%
	k__Bacteria;p__Fibrobacteres	0.11%
	k__Bacteria;p__WS2	0.06%
	k__Bacteria;p__BRC1	0.05%
	Others	0.19%

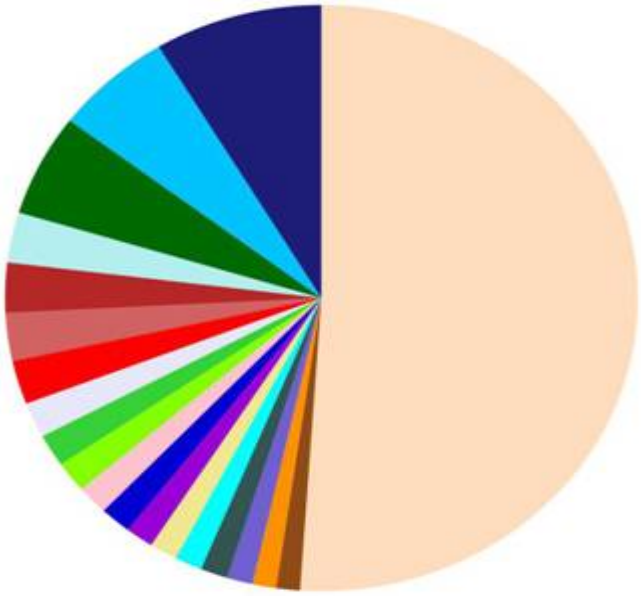
Fig. 2. Taxonomic distribution of soil bacterial population at phylum level.

bacterial population belonged to Alphaproteobacteria (21.39%), Phycisphaerae (8.93%), Saprospirae (6.81%), Actinobacteria (5.0%), Betaproteobacteria (4.38%) Deltaproteobacteria (4.02%), Bacilli (4.0%), Planctomycetia (3.66%), Thermoleophilia (3.66%), Chloracidobacteria (3.64%), Acidobacteria (3.43%), Solibacteres (3.25%), Gammaproteobacteria (2.63%), Acidimicrobiia (2.11%), Opitutae (1.98%), Chloroflexi (1.86%), Clostridia (1.77%), Nitrospira (1.53%), Gemm-1 (1.43%). A large number of bacteria (14.52%) remained unclassified at class level. At order level the bacterial population mainly belonged to WD2101 (8.6%), Shpingomonadales (7.75%), Saprospirales (6.81%), Rhizobiales (6.6%), Rhodospirillales (4.93%), Actinomycetales (4.86%), Bacillales (3.92%), Soilbacterales (3.25%), Xanthomonadales (2.43%), Myxococcales (2.3%), Acidimicrobiales (2.11%), Gemmatales (2.0%). The least abundant were Opitutales (1.97%), Solirubrobacterales (1.86%), Gaiellales (1.81%), Clostridiales (1.77%) and Roseiflexales (1.65%). At family level the largest group of bacteria (8.6%) remained unclassified under the order WD2101. For the classified ones the relative abundance was Spingomonadaceae (7.38%), Chitinophagaceae (6.76%), Rhodospirillaceae (4.18%), Bradyrhizobiaceae (2.64%), Bacillaceae (2.47%), Sinobacteraceae (2.12%), Opitutaceae (1.97%), Gemmataceae (1.85%), Hyphomicrobiaceae (1.8%), Gaiellaceae (1.79%) and Caulobacteraceae (1.48%).

At genus level the bacterial community was distributed among 19 groups (Fig. 3). The largest part (8.6%) of the bacterial community belonged to an unclassified genus from WD2101 order. The classified genera identified were those of *Flavisolibacter* (6.05%), *Kaistobacter* (5.64%), *Bacillus* (2.41%), *Opitutus* (1.95%), *Balneimonas* (1.4%), *Steroidobacter* (1.39%), *Rhodoplanes* (1.22%) and *Gemmata* (1.21%). At species level the bacterial population was largely uncategorized under different order of genus level (Fig. 4). However, based on OTU (Operational Taxonomy Unit) classification, a total number of 3123 bacterial species with Shannon alpha diversity of 9.691 could be observed in the treated soil sample.

Discussion

This study was aimed to investigate the bacterial diversity of soil which was treated with a green medium derived from the leaves of *Parthenium hysterophorus* (17). *Parthenium* residues contain various macro (N, P, K, S) and micronutrients (Fe, Mn, Zn, Cu) and (19) have observed the growth of microorganisms like Azotobacter, Actinomycetes, fungi and various phosphate solubilizing bacteria on *Parthenium* compost. Since our earlier findings (17) have also suggested the *Parthenium* derived green medium to support the growth of PGPB and therefore, here we are describing the bacterial community structure of soil in presence of this media with special emphasis on those groups having role in plant growth promotions. The microbiome profiling as carried out in this study resulted in identification of a total number of fifteen bacterial phyla i.e. Proteobacteria, Plantomycetes, Actinobacteria, Acidobacteria, Bacteroidetes, Chloroflexi, Firmicutes, Verrucomicrobia, Gemmatimonadetes, Nitrospirae, Cyanobacteria, Euryarchaeota, Armatimonadetes, Chlorobi and Fibrobacters. Most of these phyla have established roles in plant growth promotion (20). Among the major bacterial phyla detected, Proteobacteria are ubiquitously distributed in all environments and can be further classified into Classes α, β and γ Proteobacteria. They are well known for the growth promotion of cereal crops such as rice, maize, wheat, chick pea, etc (21). They also include endophytes which share symbiotic relationship with their host plant to directly or indirectly promote its growth (22). Members of this phylum are also known for the production of auxin, indole-3-acetic acid (IAA), and other important phytohormones required for the growth and development of plants (23). The phylum Actinobacteria was the third most abundant group of

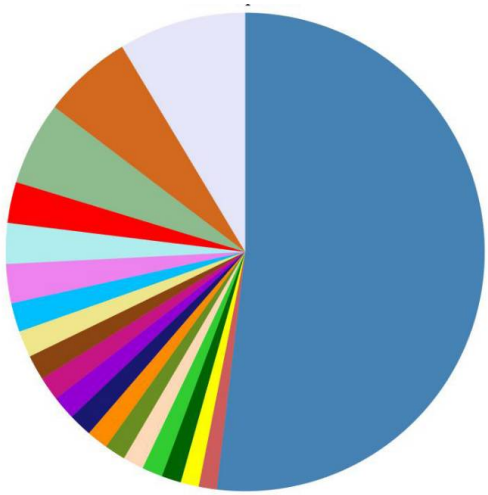


Legends	Taxonomy	Abundance
	k__Bacteria;p__Planctomycetes;c__Phycisphaerae;o__WD2101;f__Unclassified;g__Unclassified	8.6%
	k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae;g__Flavisolibacter	6.05%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Kaistobacter	5.64%
	k__Bacteria;p__Acidobacteria;c__Solibacteres;o__Solibacterales;f__Unclassified;g__Unclassified	2.77%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae;g__Unclassified	2.75%
	k__Bacteria;p__Acidobacteria;c__Acidobacteria-6;o__iii1-15;f__Unclassified;g__Unclassified	2.65%
	k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus	2.41%
	k__Bacteria;p__Verrucomicrobia;c__Opitutae;o__Opitutales;f__Opitutaceae;g__Opitutus	1.95%
	k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Gaiellales;f__Gaiellaceae;g__Unclassified	1.79%
	k__Bacteria;p__Acidobacteria;c__[Chloracidobacteria];o__RB41;f__Ellin6075;g__Unclassified	1.69%
	k__Bacteria;p__Acidobacteria;c__[Chloracidobacteria];o__RB41;f__Unclassified;g__Unclassified	1.62%
	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__MND1;f__Unclassified;g__Unclassified	1.6%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Unclassified	1.44%
	k__Bacteria;p__Gemmatimonadetes;c__Gemm-1;o__Unclassified;f__Unclassified;g__Unclassified	1.43%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae;g__Balneimonas	1.4%
	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Sinobacteraceae;g__Steroidobacter	1.39%
	k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__Unclassified;g__Unclassified	1.32%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Rhodoplanes	1.22%
	k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Gemmatales;f__Gemmataceae;g__Gemmata	1.21%
	Others	51.08%

Fig. 3. Taxonomic distribution of soil bacterial population at genus level.

bacteria found in soil treated with our green medium. Bacteria belonging to this group exhibit several plant growth promoting activities such as providing protection from fungal root pathogens by antagonism, production of lytic enzymes, antibiotics, phytohormones and siderophores as well as induction of plant defense response (24, 25). They also have ability to protect plants from harmful effect of abiotic stress by lowering the levels of ethylene by producing 1-aminocyclopropane-1-carboxylate (ACC) deaminase (24, 26). Acidobacteria is one of the most plenteous bacterial phyla of global soil environment with least known functions. Bacteria of this group are hard to culture under *in-vitro* conditions (27). Few studies indicated that they produce exopolysaccharides for adhesion on root surface and their association promotes the plant growth (28, 29).

In the present study, bacterial population of the soil treated with green medium was found to comprise bacteria belonging to the genera of *Flavisolibacter*, *Kaistobacter*, *Bacillus*, *Opitutus*, *Balneilmonas*, *Steroidobacter*, *Rhodoplanes* and *Gemmata*. Among them, *Bacillus* have the most prominent role in plant growth promotion. The endospore forming gram positive *Bacillus* are one of the most commercialized PGPB strains (30). There are reports on plant growth promoting roles of *Kaistobacter*, *Rhodoplanes*, *Balneimonas*, *Gemmata*, *Flavisolibacter* also (31–37). For example *Kaistobacter* was reported to produce



Legends	Taxonomy	Abundance
	k__Bacteria;p__Planctomycetes;c__Phycisphaerae;o__WD2101;f__Unclassified;g__Unclassified;s__Unclassified	8.6%
	k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae;g__Flavisolibacter;s__Unclassified	6.05%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Kaistobacter;s__Unclassified	5.64%
	k__Bacteria;p__Acidobacteria;c__Solibacteres;o__Solibacterales;f__Unclassified;g__Unclassified;s__Unclassified	2.77%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae;g__Unclassified;s__Unclassified	2.75%
	k__Bacteria;p__Acidobacteria;c__Acidobacteria-6;o__iii1-15;f__Unclassified;g__Unclassified;s__Unclassified	2.65%
	k__Bacteria;p__Verrucomicrobia;c__Opitutae;o__Opitutales;f__Opitutaceae;g__Opitutus;s__Unclassified	1.95%
	k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Gaiellales;f__Gaiellaceae;g__Unclassified;s__Unclassified	1.79%
	k__Bacteria;p__Acidobacteria;c__[Chloracidobacteria];o__RB41;f__Ellin6075;g__Unclassified;s__Unclassified	1.69%
	k__Bacteria;p__Acidobacteria;c__[Chloracidobacteria];o__RB41;f__Unclassified;g__Unclassified;s__Unclassified	1.62%
	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__MND1;f__Unclassified;g__Unclassified;s__Unclassified	1.6%
	k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus;s__Unclassified	1.58%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Unclassified;s__Unclassified	1.44%
	k__Bacteria;p__Gemmatimonadetes;c__Gemm-1;o__Unclassified;f__Unclassified;g__Unclassified;s__Unclassified	1.43%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae;g__Balneimonas;s__Unclassified	1.4%
	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Sinobacteraceae;g__Steroidobacter;s__Unclassified	1.39%
	k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__Unclassified;g__Unclassified;s__Unclassified	1.32%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Rhodoplanes;s__Unclassified	1.22%
	k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Gemmatales;f__Gemmataceae;g__Gemmata;s__Unclassified	1.21%
	Others	51.91%

Fig. 4. Taxonomic distribution of soil bacterial population at species level.

plant growth by producing the hormone indole-3-acetic acid (31, 32). Earlier, *Rhodoplanes* have been reported as being dominant in the organic farming system and capable of fixing atmospheric nitrogen, solubilizing phosphorus and enhancing the production of plant hormones (33).

Balneimonas belonging to phyla proteobacteria have been shown to produce IAA (35), and carry out nitrogen fixation (34). Members of this genus have been reported to be associated with an increase in enzymatic activity to degrade the organic matter and thus improve the nutrient utilization efficiency of tea plants (36).

Conclusion

An increasing trend of organic farming worldwide reflects our growing understanding towards the harmful effects of chemical fertilizers. The present study was aimed to determine the soil bacterial community structure in the presence of a dry powder, which has been earlier successfully used as a culture medium for isolation of PGPB, derived from the leaves of *Parthenium hysterophorus*. While studying the bacterial population at various taxonomic level, the abundance of bacterial groups with known PGPB role have been detected. However, the whole genome metagenomic studies need to be undertaken in future so that the presence of various genes responsible for plant growth promotions could be detected and a bacterial community metabolism favorable for plant growth could be conclusively proved.

Authors' contributions

AK did all the field and bench work and generated primary data. BR and ASP assisted in data compiling and manuscript preparation. GKJ supervised the work and prepared final manuscript with the help of AK. All authors seen the final version of the manuscript and approved the same.

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Competing interests

The authors declare that there are no competing interests.

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