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Research Article

Characterization and comparison of the plant growth promoting rhizobacteria associated with Basmati-129 & Ranbir Basmati rice indigenous to Jammu & Kashmir, India

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Abstract

Two Basmati rice cultivar (Ranbir Basmati & Basmati-129), indigenous to J&K, were explored for the plant growth promoting bacteria associated with its rhizosphere, rhizoplane and endorhizosphere during its vegetative growth stage. A total of 48 bacteria were isolated, 18 from the Ranbir Basmati cultivar (known for superior quality grain & aroma), 13 from the Basmati-129 cultivar (a high yielding variety) and 17 from the bulk field soil. The bacteria have been cataloged from rhizosphere, rhizoplane and endorhizosphere for two varieties. Sequencing of the 16S rRNA gene and plant growth promoting activities revealed that some bacteria seemed to be common to both the varieties and some were cultivar specific. However, some of these rhizobacteria were also present in the bulk soil and others were not. Therefore, both grain and the soil are source of rhizobacteria, and each cultivar selects and attracts bacteria specifically from bacterial pool present in the soil. In addition, though low number of bacterial species were isolated from the high yielding variety Basmati-129 but it harbored higher percentage of plant growth promoting rhizobacteria; PGPRs in comparison to Ranbir Basmati.

Abbreviations

RB- Ranbir Basmati; RS- Rhizosphere; RP- Rhizoplane; ER- Endorhizosphere; BS-Bulk soil

Introduction

Rice (*Oryza sativa* L.) is a vital staple crop that serves as a primary food source for a significant portion of the global population [1]. The quality of rice is determined by a combination of intrinsic and extrinsic factors. Among the extrinsic factors, pedoclimatic conditions play a significant role. The combination of soil properties (pedo) and climatic factors, affects the growth and development of rice plants, ultimately impacting the quality attributes and productivity. The major pedoclimatic factors that affect the yield are water availability, salinity, flooding, soil fertility and soil microorganisms [31, 29]. Understanding and managing these pedoclimatic factors is vital for maximizing rice production and ensuring desirable grain quality.

Out of the factors listed above, the rice rhizomicrobiome is one of the factors that plays a significant role in determining its quality [12]. The plant roots consist of three root compartments i.e., rhizosphere, rhizoplane, and endorhizosphere, hosting various microorganisms, notably bacteria. Among these microorganisms, plant growth-promoting bacteria, PGPB, have gained attention for their ability to enhance rice yield and quality. For instance, a study on one of the varieties of rice revealed PGP bacterial isolates exhibited significant increases in root length, shoot length, fresh & dry weight of roots and shoots as compared to untreated control [20]. In a separate study, two bacterial strains isolated from the rhizosphere of aromatic rice varieties; Ambemohar-157 and Dehradun Basmati were found to possess the ability to synthesize, 2-acetyl-1-pyrroline (2AP), a compound associated with rice aroma. Bacterial inoculum (single bacteria as well as bacteria consortia) led to enhancements in vegetative growth, yield, and 2AP content in comparison to the control group [11].

Basmati rice, native to Jammu in J&K, India is a distinct variety of rice, and the grains are often referred to as “scented pearls”. Understanding the microbial interactions unique to Basmati rice, can shed light on its distinct aroma and other specific characteristics. So far, only one variety of Basmati rice, Basmati-370, has been explored for PGPR associated with its rhizosphere and endorhizosphere. Diverse bacteria such as *Aeromonas hydrophila (SR18), Bacillus aryabhattai*, *B. tequilensis*, *B. subtilis, Bacillus sp., Pseudomonas mosselii, Enterobacter sp., Pseudomonas sp., and P. koreensis*

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were isolated and demonstrated significant PGP traits [20]. The present study aims to explore the cultivable diversity of PGPB associated with the rhizosphere, rhizoplane, and endorhizosphere, during the vegetative stage of two basmati varieties: Ranbir Basmati, a high-aroma-yielding variety, and Basmati-129, high yielding variety.

Material & Methods

Sample collection and bacterial isolation

Soil and root samples of locally cultivated basmati rice plants i.e., Ranbir Basmati and Basmati-129 were collected during the vegetative stage from the field of SKUAST, Chatha district of Jammu (32.44 °N and 74.54 °E) in the year 2018. Roots were cut and placed in 1X phosphate buffered saline solution, PBS (10 mM PO43−, 137 mM NaCl, and 2.7 mM KCl) with pH 7.4 till processed for root compartment separations i.e., rhizosphere, rhizoplane, and endorhizosphere [14]. Bulk soil; unplanted soil from the field was gathered from a depth of 0 to 20 cm, to serve as a reference [47].

The roots were washed with PBS that was subsequently used for the isolation of for rhizosphere bacteria. For isolation of rhizoplane bacteria, the washed roots were transferred to fresh sterile PBS buffer and sonicated thrice at 50-60 Hz for 30 sec in a water bath. For the isolation of endorhizosphere bacteria, the washed roots were surface sterilized using 70% ethanol for 5 minutes, 1.2% (v/v) sodium hypochlorite for 15 minutes, and finally washing with sterile distilled water six times [5]. The endorhizosphere was collected by crushing the sterilized roots in PBS using an autoclaved mortar and pestle.

Following root zone separation, the PBS buffer of each compartment was serially diluted, and 100 µL aliquots were plated on sterile nutrient agar plates in triplicate. In addition, 100µL of the final wash of surface sterilization step was plated on the sterile NA plates and observed for the growth of any rhizoplane bacteria before maceration. The plates were then incubated in an incubator (Scigenics; ORBITEK LEBT) at a temperature of 28±2°C for two days to determine the count of bacterial colony units i.e., CFUs.

Identification of bacteria for plant growth promoting properties

Bacterial isolates were screened for the plant growth promotion activities, such as phosphate solubilization, indole acetic acid production, ammonia generation, and siderophore production, as well as cellulase, catalase, protease, and amylase enzymatic activities. Phosphate solubilization by the bacterial isolates was evaluated by growing the isolates on Pikovskaya's agar medium containing tricalcium phosphate and incubating for 4-5 days at 28 °C. Clear zones surrounding the bacterial colonies indicated phosphate solubilization [39]. The synthesis of indole acetic acid was estimated by growing the bacteria in LB medium containing 100 µg/mL of tryptophan at 37 ^{0}C for 48 hours followed by centrifugation at 12,000 rpm for 5 minutes. To assess indole acetic acid (IAA) production, 2 mL of Salkowski's reagent (1 ml of 35% perchloric acid and 49 ml of 0.5 M ferric chloride) was added to 1 ml of the supernatant and incubated at 25°C for 30 minutes for the production of pink colour, followed by measuring the absorbance at 530 nm using a UV/visible spectrophotometer (Thermoscientific; Genesys). Pure indole acetic acid (IAA) was used as positive control [34]. The isolates' ability to produce ammonia was evaluated according to Cappuccino and Sherman's protocol [9]. Bacterial cultures grown overnight were inoculated in 10 mL of peptone water and then incubated for 48 hours at 28 °C in a shaker incubator (Scigenics; ORBITEK LEBT). Following incubation, 0.5 mL of Nessler's reagent was added. The transition from a light yellow to a dark brown color indicated ammonia production. Chrome Azurol-S medium (CAS medium) was used to screen for the siderophore synthesis [2]. The CAS agar medium was streaked with bacterial isolates, and then incubated at 28 °C for 3–5 days. According to Alexander and Zuberer [2], the appearance of a yellow-orange halo zone around the culture serves as an indicator of siderophore production. Bacterial isolates were inoculated on different substrate media such as: starch agar media for amylase test [17], carboxymethylcellulose agar media for cellulase test [10], skim milk agar media for protease activity [41], and then incubated at 37°C for 24 hours. Amylase activity was indicated by the appearance of a clear zone around the bacterial colonies. Iodine-based clear zones served as a signal for the cellulase activity test. A distinct zone surrounding colonies indicated positive protease activity. Catalase test was performed by mixing the bacterial colony with 3% hydrogen peroxide on a glass slide [36], and a positive catalase activity was demonstrated by the effervescence.

Identification of bacterial isolates by 16S rRNA gene sequencing

The isolated and purified bacteria were identified based on their morphological and molecular phylogeny analysis. Colony PCR amplification of these bacterial isolates was performed with certain modifications of method developed by Woodman et al [48]. A portion of overnight-grown colony was picked with a tip of a sterile toothpick and resuspended in the 20 µl of autoclaved MiliQ. The tubes were vortexed for 30 seconds, and cell wall lysis was done by placing the tubes in the microwave at the highest temperature for 2 minutes followed by vortexing for 30 seconds. The tubes were centrifuged for 2 minutes at 13,000 rpm at 4^{0}C and the supernatant was used as template DNA. The 16S rRNA gene was amplified from the genomic DNA, using universal 16S rRNA gene primers [18]. Sequencing of the 16S rRNA gene amplicons was conducted at Agri-Genome Labs, Chennai, India. The nucleotide sequences

were analyzed for taxonomical affiliation using the BLASTn analysis against the NCBI's nucleotide sequence database (nr database http://www.ncbi.nlm.nih.gov). The sequences were submitted to the GenBank nucleotide sequence database under accession no MN653270- 82, MN653314-20, MN653360-76, MN704775-94. Taxonomical affiliation of the isolated strains along with its accession numbers has been represented in Tables 2 and 3.

In order to gain further insights into the phylogenetic relationships among the isolated bacterial strains, sequence alignment of the 16S rRNA gene sequences was performed using BioEdit sequence aligner was performed [15]. Subsequently, a neighbor-joining tree was constructed using MEGA software, with a bootstrap value of 1000 to assess the robustness of the tree topology [23]

Results

Bacterial isolation from different compartments of vegetative stage

Bacteria were isolated from the rhizosphere, rhizoplane, and endorhizosphere of both the cultivars i.e., Ranbir Basmati and Basmati-129 rice (Figs. 1 and 2). A total of 48 bacterial isolates were obtained from both the basmati rice varieties, 18 from the Ranbir Basmati cultivar, 6 from the rhizosphere, 7 from the rhizoplane, and 5 from the endorhizosphere. A total of 13 bacteria were isolated from Basmati-129 with 3 rhizospheres, 5 each from rhizoplanes, and endorhizosphere. In addition, 17 bacteria were isolated from the bulk soil.

In the case of Ranbir Basmati, the bacterial load in the rhizosphere, rhizoplane, and endorhizosphere was $7.8*10^5$ CFU/g, $0.49*10^5$ CFU/g, and $4.4*10^5$ CFU/g, respectively, whereas in the case of Basmati-129, the bacterial load was $34*10^5$ CFU/g, $0.29*10^5$ CFU/g, and $0.78*10^5$ CFU/g in the rhizosphere, rhizoplane, and endorhizosphere, respectively. On the other hand, the bacterial load in bulk soil was $4.5*10^5$ CFU/g (Fig. 3).

Identification of isolates using 16S rRNA sequencing

The analysis of 16S rRNA gene sequencing uncovered a diverse microbial community comprising thirteen distinct genera: *Bacillus, Priestia, Cytobacillus, Peribacillus, Enterobacter, Pseudomonas, Brevibacillus, Fictibacillus, Exiquobacterium, Agrobacterium, Brevibacterium, Stenotrophomonas,* and *Rhizobium*. In the case of Ranbir Basmati, bacteria including *Peribacillus huizhouensis, B. paramycoides, , B. pacificus, B. thaohiensis, Priestia megaterium* and *Enterobacter bugandensis* were present in the rhizosphere. The rhizoplane was inhabited by *Pseudomonas corrugata, Brevibacillus agri, Br. parabrevis, Bacillus anthracis*, and *Fictibacillus phosphorivorans*. In the endorhizosphere, the interior of the root system, *Bacillus zhangzhouensis, Priestia megaterium, Fictibacillus halophilus,* and *Exiquobacterium acetylicum* exhibited prominence. However, in the case of Basmati-129, *Bacillus velezensis, Priestia flexa* and *Pseudomonas migulae* were found in the rhizosphere, whereas the bacteria *Exiquobacterium acetylicum, Bacillus subtilis, Brevibacillus reuszeri, Pseudomonas chengduensis,* and *P. oleovorans sub. lubricants* were isolated from the rhizoplane. Further, *Bacillus paramycoides, B. albus, B. paramycoides, Priestia aryabhattai,* and *Stenotrophomonas pavanii,* were found in the endorhizosphere. As expected, the bulk soil had highest bacterial diversity, harboring *Bacillus sp., Fictibacillus phosphorivorans, Bacillus thuringiensis, B. velezensis, B. cereus, Priestia megaterium, Cytobacillus firmus, Metabacillus indicus, Fictibacillus barbaricus, Agrobacterium larrymoorei, A. tumefaciens, Peribacillus frigoitolerans,* and *Rhizobium sp*. It was observed that *Bacillus* was the dominant genera not only in the bulk soil but across the root compartments of both the cultivars. The comparative presence of bacterial species across various compartments and bulk soil in both Ranbir Basmati and Basmati-129 rice varieties is summarized in Figs. 4 and 5. Bacterial diversity has also been represented in the form of heatmap in Fig. 6 that highlights the common bacterial species present among these compartments within each variety. A comparison of common bacteria across different compartments and their 16S rRNA gene sequence similarities is provided in Table 1. This provides insights into the microbial composition and distribution within the rice plant root ecosystem. Bacterial diversity has also been represented in the form of heatmap in Fig. 6. The neighbor joining phylogenetic tree of all the isolates has been represented in Supplementary file.

Screening of bacterial isolates for plant growth promoting (PGP) characteristics

Isolates from both varieties were screened for eight plant-growth-promoting activities, i.e., phosphate solubilization, indole acetic acid, ammonia, and siderophore production, in addition to cellulase, catalase, protease, and amylase activity (Fig. 6). All the bacterial isolates from both the rice varieties produced indole acetic acid and siderophore. Phosphate solubilization was exhibited by the least percentage of isolates in both varieties; however, it was comparatively high in the case of Basmati-129. Among different compartments of Ranbir Basmati, a high percentage of phosphate solublizing and ammonia-producing bacteria were found in the rhizoplane and endorhizosphere, whereas a high percentage of cellulase- and amylase-producing bacteria were found in the rhizosphere. Rest all the activities were equally exhibited by the rhizobacteria of all the three compartments. On the other hand, among different compartments of Basmati-129, a high percentage of cellulase, catalase, and amylase-producing bacteria were found in the rhizosphere and rhizoplane, whereas phosphate- and protease-producing bacteria were found in a high percentage in the rhizoplane

whereas phosphate producers were less in endorhizosphere. It was also noted that in both the varieties, the percentage of plant growth-promoting bacteria in bulk soil was comparatively lesser than the root compartments.

Discussion

The selection of the two Basmati rice varieties, Ranbir Basmati and Basmati-129, for this study holds significance due to their distinct characteristics and regional importance. Ranbir Basmati has deep roots in the culture and farming practices of Jammu and Kashmir. It's a traditional rice type known for good-quality grains that have a distinct aroma. It takes around 120 days to be ready for harvest, which is 20–30 days earlier than another popular variety, Basmati-370 [35]. On the other hand, Basmati-129, which also comes from the same region, has a short stature and weak aroma but is a high-yielding variety [7]. Studying the vegetative stage of rice is important as this phase involves active root-microbe interactions, nutrient exchange, and the exudation of compounds that shape the surrounding soil's microbial community [28]. The rhizosphere, positioned near the roots, serves as a hotspot environment for microbial activity due to the release of root exudates, creating a unique microbial community composition compared to the bulk soil [30, 38]. Rhizoplane, acts as a gateway for determining the entry of microbes into the host root tissue and influencing their establishment and activities [14, 45]. The endorhizosphere, the inner root tissue, have distinct microbiome with potential benefits for plant growth, stress tolerance, and defense against pathogens. [8, 37, 16].

Even though, less number of bacteria were isolated from the rhizosphere of Basmati-129 as compared to Ranbir Basmati, both the cultivars have specific strategies for adapting and interacting with its environment and selecting specific bacteria from bacterial pool present in the soil (Fig. 4). This is also evident from the bacterial load in the rhizosphere and rhizoplane that is lesser in Basmati-129 than Ranbir Basmati. Though roots of both the varieties, as in case of other plants, must be releasing specific root exudates that contain chemical signals that attract and repel certain microbial species, creating a microbial community tailored to its specific needs [4]. Specific microbial species facilitate the mobilization and solubilization of essential nutrients, such as nitrogen and phosphorus, making them more readily available to the rice plant [32].

The bacterial load in the root compartments is reported to decrease sequentially from rhizosphere to rhizoplane to endorhizosphere [8, 16, 14]. In both varieties, we have found a decrease in bacterial load from rhizosphere to endorhizosphere (Fig. 3). This concept aligns with a study of two high-yield rice cultivars in Venezuela wherein the bacterial load of rhizosphere (5.5×10^7 CFU/g) was higher as compared with endorhizosphere (121,076 CFU/g) [5]. Similarly, a study on Jammu Basmati 370 grown in Chohalla, Ranbir Singh Pura revealed higher bacterial counts in rhizosphere (3.3×10^6 CFU/g) as compared to endorhizosphere (1.4×10^5 CFU/g) [20]. However, the bacterial load in rhizoplane was not studied in these two reports [5,20]. Our results show a departure from the expected pattern of a decline in the microbial load from the rhizosphere to the rhizoplane, followed by the endorhizosphere as there is relatively higher bacterial load in the endorhizosphere than the rhizoplane that needs to be reevaluated in future studies.

Selection of specific bacteria by each rice cultivar was evident as out of 17 bulk soil bacteria, only *Bacillus velezensis* was present in Basmati-129 whereas only two bacteria namely *Bacillus thaohiensis* and *Priestia megaterium* were present in Ranbir Basmati. This observation has been made on the basis of similar 16S rRNA gene sequences and PGP activities of these bacterial isolates (Fig. 6 and Table 1). Presence of specific bacteria in different rice varieties may be indicative of a process governing the migration of rhizobacteria from the bulk soil to the interior of roots that in turn is regulated by a complex interplay of both active and passive movement mechanisms [6]. This is attributed to their initial colonization of the bulk soil, driven by their ability to metabolize root exudates. Over time, these bacteria adapt and move inward, taking advantage of the nutrient-rich environment within the rhizosphere and endorhizosphere. This observation is supported by a study in which researchers explored the root microbiota of *Arabidopsis thaliana*. They found that bacteria capable of metabolizing root exudates thrive in the rhizosphere. Over time, interactions between roots and microbes facilitate the migration of these bacteria inward, shaping the distinct distributions observed in different compartments [8].

Surprisingly, two out of the three rhizospheric bacteria in Basmati-129 - *Pseudomonas migulae* and *Priestia flexa* were not isolated from the bulk soil. Similarly, *Bacillus paramycoides*, *Peribacillus huizhouensis*, *Bacillus pacificus,* and *Enterobacter bugandensis* in Ranbir Basmati present in the rhizosphere are absent in bulk soil. These bacteria are known for their various plant-growth promoting activities [19, 24, 49, 46, 22]. This phenomenon can be supported by a similar study conducted on Basmati-370, where some of the rhizospheric bacteria were absent in the bulk soil [20]. This suggests that the selective presence of beneficial rhizobacteria in the rhizosphere is a consistent phenomenon in Basmati rice varieties. The source of these bacteria is a matter of further investigation.

The presence of *Exiquobacterium acetylicum* in the endorhizosphere of Ranbir Basmati and the rhizoplane of Basmati-129, but its absence in bulk soil, suggests it may be seed-borne and helpful in growth promotion of the plant as it exhibited all the screened PGP activities. The ability of *Exiquobacterium acetylicum* to enhance nutrient availability through processes such as nitrogen fixation, phosphate solubilization, and IAA production is

known to contribute to plant growth and has been reported in other plants as well [3, 42].

We found that *Priestia megaterium*, known for its plant growth-promoting (PGP) abilities [50], was present in the bulk soil but also in the rhizosphere and endorhizosphere of Ranbir Basmati and possessed all the PGP activities except phosphate solubilization. *Priestiamegaterium* has been previously reported from rice rhizosphere in Northeast China with PGP ability such as potassium and phosphorus solubilization and Indole-3-acetic acid, Gibberellic acid, and siderophores production and promoted the growth of rice seedlings in pot assays [25]. Its absence from the rhizosphere of the Basmati-129. confirms that this bacterium has been selected by Ranbir basmati and mechanism of its association is the matter of further study.

This analysis can be visualized through heatmap also wherein three distinct clades were formed (Fig 7). The bulk soil microorganisms formed a clade with Basmati-129 rhizospheric microbes that is evident by more number of common bacteria. The rhizoplane of Basmati-129 and the endorhizosphere of Ranbir Basmati seemed to closely associate and were grouped together. A similar connection was observed between the endorhizosphere of Basmati-129 and the rhizosphere of Ranbir Basmati and they were interconnected through the shared microbial community in the rhizoplane of Ranbir Basmati. This microbial network again signifies the interconnected nature of microbial communities within different compartments and bulk soil of these two rice varieties.

In our study, the *Bacillus* genus was dominant in both the varieties as well as in the bulk soil. In Ranbir Basmati, it accounted for 50% of the isolates, and in Basmati-129, it constituted 53.85% of the isolates whereas bulk soil represented a substantial 64.71% of the *Bacillus* isolates. These findings align with previous studies that have shown the prevalence of *Bacillus* genus, in various crop systems [40, 33, 21]. Plant roots have been reported to recruit multiple bacteria with similar traits, a strategy known as functional redundancy. This ensures that essential plant-microbe interactions persist even in the face of competition or environmental stress. These microbes complement each other's functions, adapt to diverse rhizosphere niches, enhance ecosystem stability, and collectively defend against pathogens [26, 27, 30, 44].

Furthermore, the 16S rRNA sequence similarity, which examines a specific genetic marker known as the 16S ribosomal RNA (rRNA) gene, within the bacteria shared across our study, indicates a high degree of genetic similarity (Table 1). The neighbor-joining tree further supports our findings, showing that the bacteria found common in our study have relatively less genetic differentiation if any. (Supplementary file)

The presence of specific bacterial species in the high-yielding Basmati-129 variety, along with their demonstrated plant growth-promoting activities, suggests a potential correlation between these factors and the observed high yield. These bacteria contribute to improved nutrient availability, stress tolerance, and overall plant health, ultimately enhancing the yield of Basmati-129. Conversely, the presence of specific bacterial species in Ranbir Basmati, coupled with their relatively lower plant growth-promoting activities, partly explains the comparatively lower yield of this variety. Among the activities analyzed, phosphate solubilization and amylase activity was exhibited by more bacteria in Basmati-129 than Ranbir Basmati (Table 4 and 5). Phosphate solubilizing activity aids in the conversion of insoluble phosphates into forms that plants can readily absorb, thereby improving phosphorus uptake and overall nutrient availability [43]. Amylase activity, on the other hand, is crucial for the efficient breakdown of starch into simpler sugars, facilitating enhanced energy production for plant growth [13]. Interestingly Basmati-129 has recruited lesser bacteria comparatively but all possess PGP activities whereas bacteria that are recruited by Ranbir Basmati show less PGP activities (Table 4 and 5). The association of bacteria having less PGP activity with Ranbir basmati could be one of the reasons for its lower yield. This can be supported by a study wherein they applied 18 most promising native strains of PGPR, either alone or in combination with 50% of the recommended dosage of NPK fertilizers (RDF) to assess their impact on the morphological growth parameters of rice and demonstrated an increase in plant growth compared to the control [20].

Future course in this study is the identification of aroma enhancing PGP bacteria from Ranbir Basmati and design a synthetic microbial community with aroma enhancing and yield increasing bacteria from Basmati-129 and their in-field evaluation on both the varieties.

Conclusion

The two basmati rice varieties, Ranbir Basmati with superior grain & aroma quality and Basmati-129 with high yield were compared for bacterial diversity and function in various root compartments. It was observed that each harbored unique bacteria and also a few common bacteria, i.e.; *Priestia megaterium*, *B. thaohiensis* and *B. velezensis*. However, source of these bacteria could be either bulk soil or grain, which needs further investigation. Interestingly. the high yielding variety Basmati-129 harbored fewer bacteria (13) in comparison to Ranbir Basmati (18) but most of the root associated bacteria from Basmati-129 had more PGP activities as compared to other Basmati variety. These results co-relate the presence of PGPR to higher yield. In future, Ranbir Basmati can be bioaugmented with various combinations of bacteria isolated from Basmati-129 to study their effect on its yield.

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Declarations

Conflict of interest

All authors declare that there is no conflict of interest.

Author contributions

Jyoti Vakhlu conceptualized the research project, secured funding, and reviewed the manuscript; Tabia Andrabi and Nitika Sharma experimented and drafted the whole manuscript; Sheetal Ambardar supervised bioinformatics analysis and reviewed the manuscript; R.K. Salgotra designed the experiment and reviewed the manuscript. All the authors read and approved the final manuscript.

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Figures

Fig 1: Site for sample collection

Fig 2: Root harvesting and root compartments separation A. Rhizosphere B. Rhizoplane C. Endorhizosphere

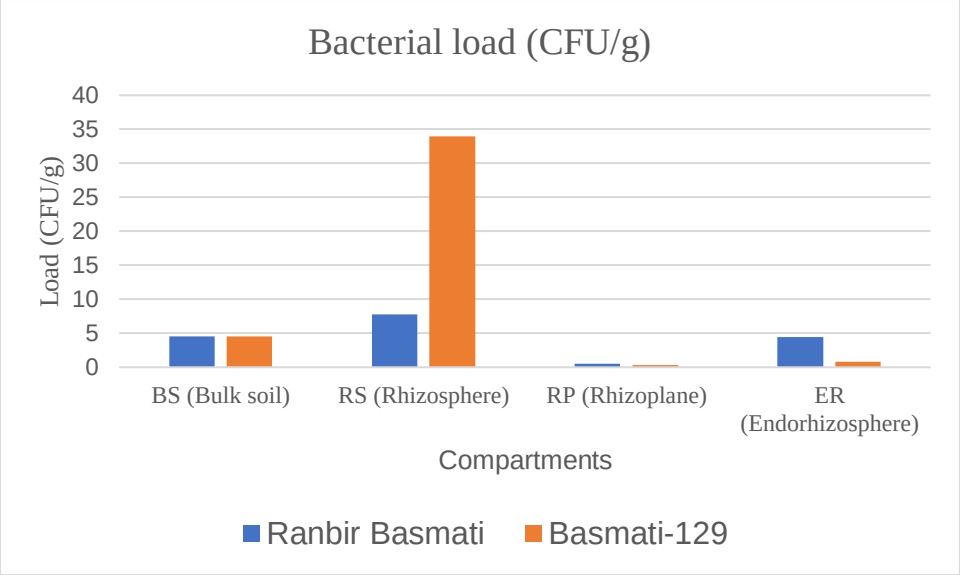

Fig 3: Bacterial load as determined in different compartments of vegetative stage in Ranbir Basmati and Basmati-129 varieities (CFU/g: Colony forming units per gram; BS: Bulk soil; RS: Rhizosphere; RP: Rhizoplane; ER: Endorhizosphere)

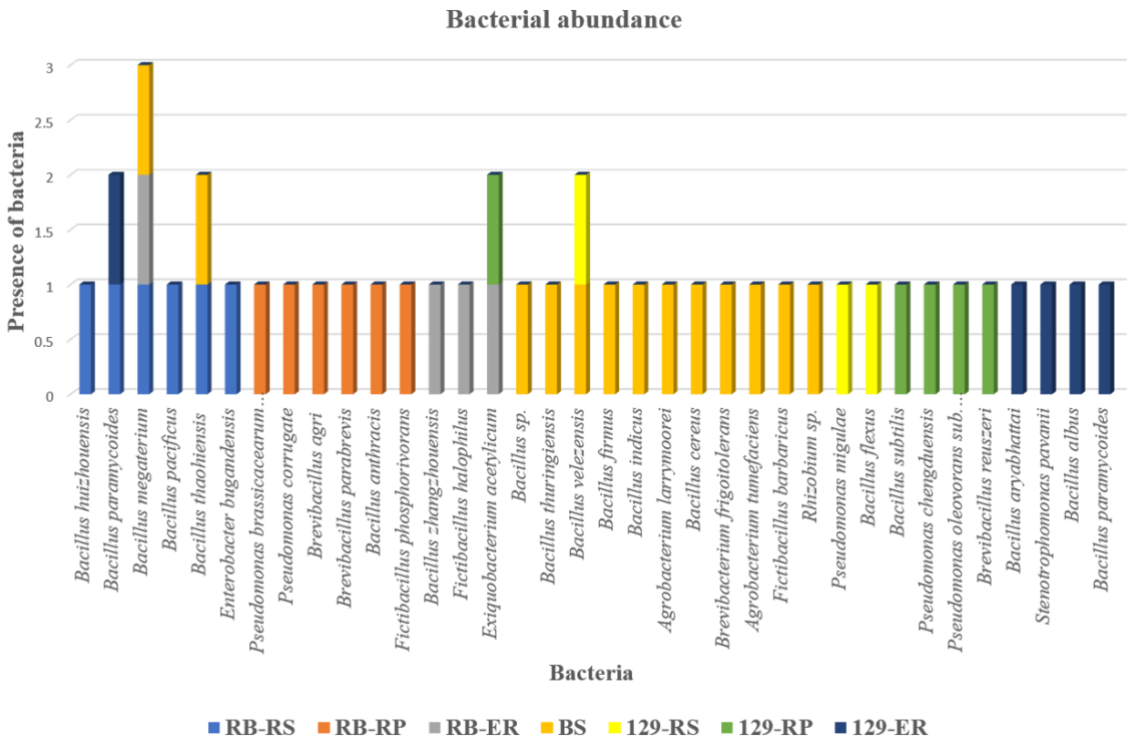

Fig 4: Comparison of bacterial abundance in different compartments of Ranbir Basmati and Basmati-129 rice varieties (RB-RS: Ranbir Basmati-Rhizosphere; RB-RP: Ranbir Basmati-Rhizoplane; RB-ER: Ranbir Basmati-Endorhizosphere; BS: Bulk soil; 129-RS: Basmati 129-Rhizosphere; 129-RP: Basmati 129-Rhizoplane; 129-ER: Basmati 129-Endorhizosphere)

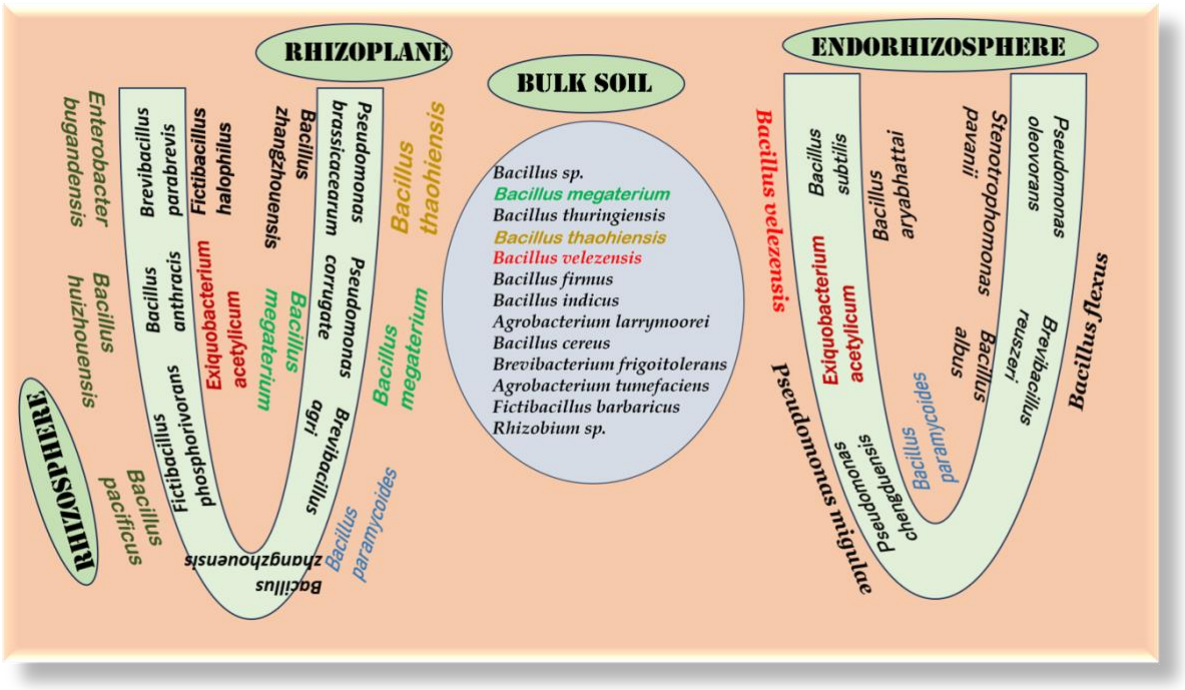

Fig 5: Pictographic comparison of bacterial abundance in different compartments of Ranbir Basmati and Basmati-129 rice varieties

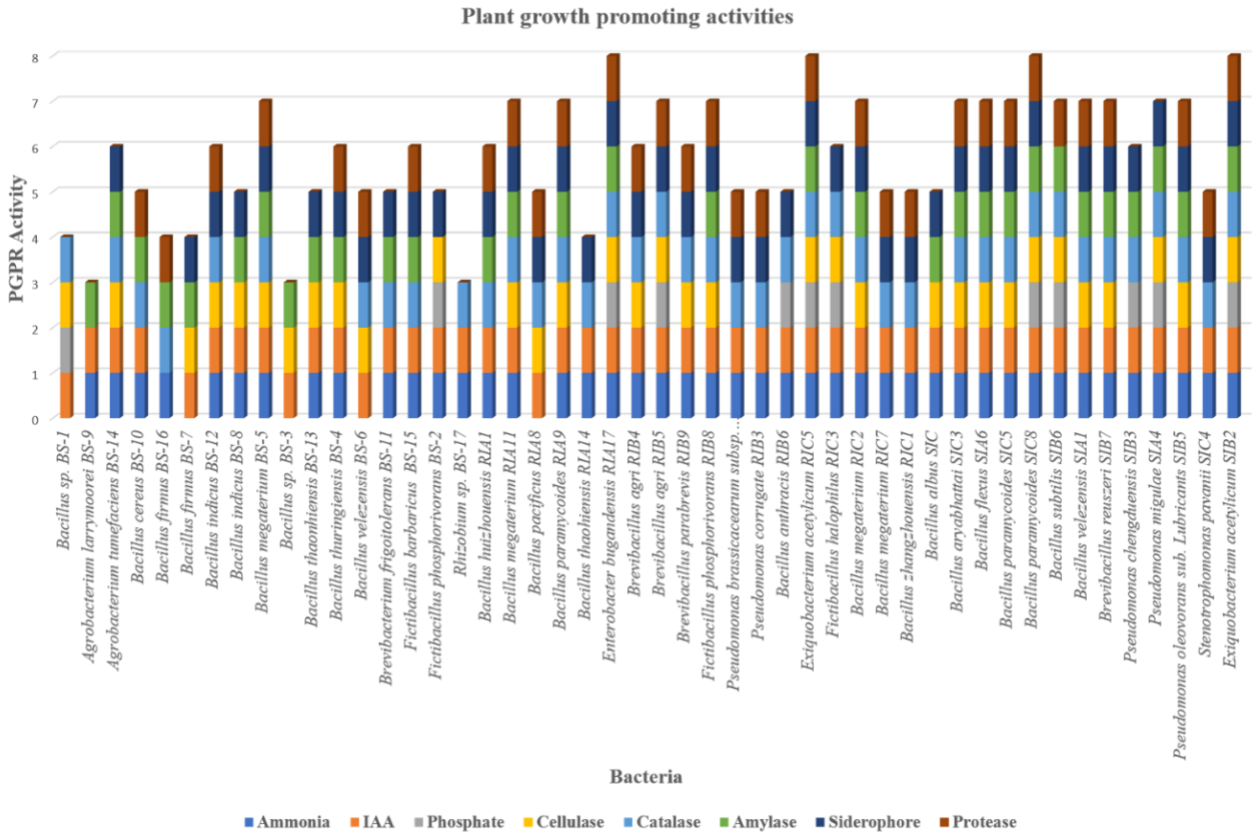

Fig 6: Plant-growth promoting activities exhibited by microbial diversity

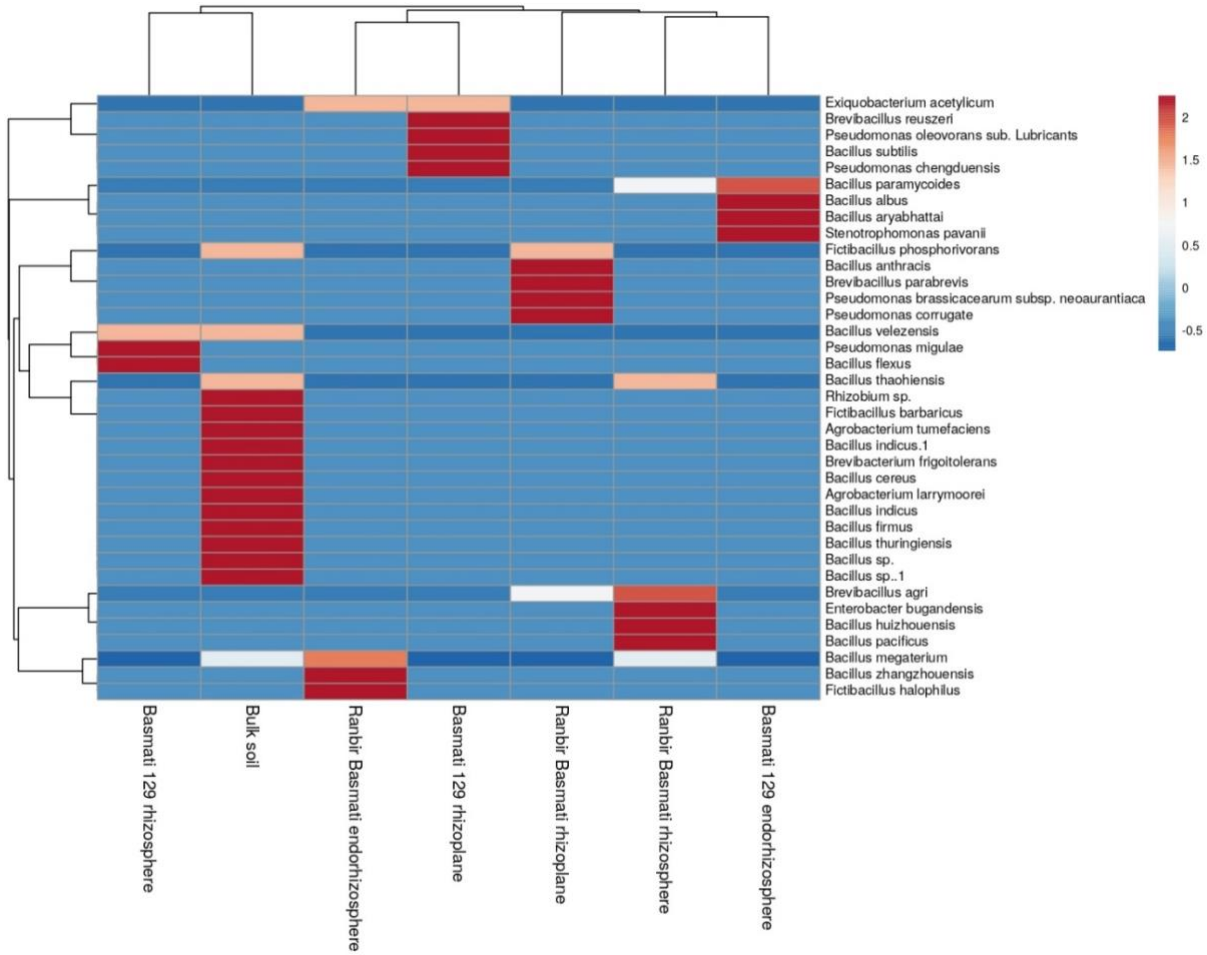

Fig 7: Hierarchical clustering heatmap of microbial diversity across different compartments of two varieties (https://biit.cs.ut.ee/clustvis/)

Tables

Table 1: Genetic similarity and compartment distribution of common bacterial isolates based on 16S rRNA gene sequences (RB-RS: Ranbir Basmati- Rhizosphere; RB-ER: Ranbir Basmati-Endorhizosphere; BS: Bulk soil; 129-RP: Basmati 129- Rhizoplane)

Common bacteria	Common compartments	16S rRNA gene sequence similarity
Priestia megaterium	RB-RS	99.1%
	RB-ER	
	BS	
Bacillus thaohiensis	RB-RS	97.8%
	BS	
Exiquobacterium acetylicum	RB-ER	99.0%
	129-RP	

Common bacteria	Common compartments	16S rRNA gene sequence similarity
Bacillus velezensis	BS	98.3%
	129-RS	
Bacillus paramycoides	RB-RS	98.9%
	129-ER	

Table 2: Molecular identification of different root compartment isolates from Ranbir Basmati (RI-A: Rhizosphere; RI-B: Rhizoplane; RI-C: Endorhizosphere; BS: Bulk soil)

Isolate ID	Identification based on 16S rRNA sequencing	Root zone	% Similarity	GenBank Accession number of identified bacteria	Link to GenBank nucleotide sequence database
RI-A1	*Peribacillus huizhouensis* strain RI-A1	Rhizosphere	93.35	MN653270	https://www.ncbi.nlm.nih.gov/search/all/?term=MN653270
RI-A9	*Bacillus paramycoides* strain RI-A9		100	MN704776	https://www.ncbi.nlm.nih.gov/search/all/?term=MN704776
RI-A11	*Priestia megaterium* strain RI-A11		97.12	MN653271	https://www.ncbi.nlm.nih.gov/search/all/?term=MN653271
RI-A8	*Bacillus pacificus* strain RI-A8		99.26	MN704775	https://www.ncbi.nlm.nih.gov/search/all/?term=MN704775
RI-A14	*Bacillus thaonhiensis* strain RI-A14		97.41	MN653272	https://www.ncbi.nlm.nih.gov/search/all/?term=MN653272
RI-A17	*Enterobacter bugandensis* strain RI-A17		92.03	MN653273	https://www.ncbi.nlm.nih.gov/search/all/?term=MN653273
RI-B2	*Pseudomonas brassicacearum subsp. neoaurantiaca* strain RI-B2	Rhizoplane	97.10	MN653274	https://www.ncbi.nlm.nih.gov/search/all/?term=MN653274
RI-B3	*Pseudomonas corrugata* strain RI-B3		94.32	MN653275	https://www.ncbi.nlm.nih.gov/search/all/?term=MN653275
RI-B4	*Brevibacillus agri* strain RI-B4		97.92	MN704777	https://www.ncbi.nlm.nih.gov/nuccore/MN704777

Isolate ID	**Identification based on 16S rRNA sequencing**	**Root zone**	**% Similarity**	**GenBank Accession number of identified bacteria**	**Link to GenBank nucleotide sequence database**
RI-B5	*Brevibacillus agri* strain RI-B5		97.58	MN653276	Brevibacillus agri strain RI-B5 16S ribosomal RNA gene, partial sequen - Nucleotide - NCBI (nih.gov)
RI-B9	*Brevibacillus parabrevis* strain RI-B9		98.05	MN704778	https://www.ncbi.nlm.nih.gov/nuccore/MN704778
RI-B6	*Bacillus anthracis* strain RI-B6		99.77	MN653277	https://www.ncbi.nlm.nih.gov/nuccore/MN653277
RI-B8	*Fictibacillus phosphorivorans* strain RI-B8		99.89	MN653278	https://www.ncbi.nlm.nih.gov/nuccore/MN653278
RI-C1	*Bacillus zhangzhouensis* strain RI-C1	Endorhizosphere	99.66	MN653279	https://www.ncbi.nlm.nih.gov/nuccore/MN653279
RI-C2	*Priestia megaterium* strain RI-C2		99.37	MN653280	https://www.ncbi.nlm.nih.gov/nuccore/MN653280
RI-C3	*Fictibacillus halophilus* strain RI-C3		99.33	MN653281	https://www.ncbi.nlm.nih.gov/nuccore/MN653281
RI-C5	*Exiguobacterium acetylicum* strain RI-C5		99.75	MN653282	https://www.ncbi.nlm.nih.gov/nuccore/MN653282
RI-C7	*Priestia megaterium* strain RI-C7		98.32	MN704779	https://www.ncbi.nlm.nih.gov/nuccore/MN704779
BS-1	*Bacillus sp. (in: firmicutes)* strain BS-1	Bulk Soil	99.44	MN653360	https://www.ncbi.nlm.nih.gov/nuccore/MN653360
BS-2	*Fictibacillus phosphorivorans* strain BS-2		99.43	MN653361	https://www.ncbi.nlm.nih.gov/nuccore/MN653361
BS-3	*Bacillus sp. (in: firmicutes)* strain BS-3		98.52	MN653362	https://www.ncbi.nlm.nih.gov/nuccore/MN653362

Isolate ID	Identification based on 16S rRNA sequencing	Root zone	% Similarity	GenBank Accession number of identified bacteria	Link to GenBank nucleotide sequence database
BS-4	*Bacillus thuringiensis* strain BS-4		100	MN653363	https://www.ncbi.nlm.nih.gov/nuccore/MN653363
BS-5	*Priestia megaterium* strain BS-5		100	MN653364	https://www.ncbi.nlm.nih.gov/nuccore/MN653364
BS-6	*Bacillus velezensis* strain BS-6		100	MN653365	https://www.ncbi.nlm.nih.gov/nuccore/MN653365
BS-7	*Cytobacillus firmus* strain BS-7		100	MN653366	https://www.ncbi.nlm.nih.gov/nuccore/MN653366
BS-8	*Metabacillus indicus* strain BS-8		99.87	MN653367	https://www.ncbi.nlm.nih.gov/nuccore/MN653367
BS-9	*Agrobacterium larrymoorei* strain BS-9		99.88	MN653368	https://www.ncbi.nlm.nih.gov/nuccore/MN653368
BS-10	*Bacillus cereus* strain BS-10		100	MN653369	https://www.ncbi.nlm.nih.gov/nuccore/MN653369
BS-11	*Peribacillus frigoritolerans* strain BS-11		99.78	MN653370	https://www.ncbi.nlm.nih.gov/nuccore/MN653370
BS-12	*Metabacillus indicus* strain BS-12		99.89	MN653371	https://www.ncbi.nlm.nih.gov/nuccore/MN653371
BS-13	*Bacillus thaonhiensis* strain BS-13		99.65	MN653372	https://www.ncbi.nlm.nih.gov/nuccore/MN653372
BS-14	*Agrobacterium tumefaciens* strain BS-14		100	MN653373	https://www.ncbi.nlm.nih.gov/nuccore/MN653373
BS-15	*Fictibacillus barbaricus* strain BS-15		99.63	MN653374	https://www.ncbi.nlm.nih.gov/nuccore/MN653374
BS-16	*Cytobacillus firmus* strain BS-16		99.55	MN653375	https://www.ncbi.nlm.nih.gov/nuccore/MN653375

Isolate ID	Identification based on 16S rRNA sequencing	Root zone	% Similarity	GenBank Accession number of identified bacteria	Link to GenBank nucleotide sequence database
BS-17	*Rhizobium sp.* strain BS-17		99.88	MN653376	https://www.ncbi.nlm.nih.gov/nuccore/MN653376

Table 3: Molecular identification of different root compartment isolates from Basmati-129 (SI-A: Rhizosphere; SI-B: Rhizoplane; SI-C: Endorhizosphere)

Isolate ID	Identification based on 16S rRNA sequencing	Root zone	% Similarity	GenBank Accession number identified bacteria	Link to GenBank nucleotide sequence database
SI-A1	*Bacillus velezensis* strain SI-A1	Rhizosphere	98.37	MN704793	https://www.ncbi.nlm.nih.gov/nuccore/MN704793
SI-A4	*Pseudomonas migulae* strain SI-A4		100	MN704789	https://www.ncbi.nlm.nih.gov/nuccore/MN704789
SI-A6	*Priestia flexa* strain SI-A6		95.26	MN653314	https://www.ncbi.nlm.nih.gov/nuccore/MN653314
SI-B2	*Exiguobacterium acetylicum* strain SI-B2	Rhizoplane	100	MN704794	https://www.ncbi.nlm.nih.gov/nuccore/MN704794
SI-B6	*Bacillus subtilis* strain SI-B6		99.25	MN704790	https://www.ncbi.nlm.nih.gov/nuccore/MN704790
SI-B3	*Pseudomonas chengduensis* strain SI-B3		99.23	MN653315	https://www.ncbi.nlm.nih.gov/nuccore/MN653315
SI-B5	*Pseudomonas oleovorans subsp. lubricantis* strain SI-B5		99.86	MN653316	https://www.ncbi.nlm.nih.gov/nuccore/MN653316
SI-B7	*Brevibacillus reuszeri* strain SI-B7		92.68	MN653317	https://www.ncbi.nlm.nih.gov/nuccore/MN653317
SI-C3	*Priestia aryabhattai* strain SI-C3	Endorhizosphere	94.72	MN653320	https://www.ncbi.nlm.nih.gov/nuccore/MN653320
SI-C5	*Bacillus paramycoides* strain SI-C5		99.2	MN704792	https://www.ncbi.nlm.nih.gov/nuccore/MN704792
SI-C4	*Stenotrophomonas pavanii* strain SI-C4		99.89	MN653318	https://www.ncbi.nlm.nih.gov/nuccore/MN653318
SI-C	*Bacillus albus* strain SI-C		100	MN704791	https://www.ncbi.nlm.nih.gov/nuccore/MN704791

SI-C8	*Bacillus paramycoides* strain SI-C8		99.65	MN653319	https://www.ncbi.nlm.nih.gov/nuccore/MN653319

Table 4: Numbers of bacteria exhibiting various plant growth promoting activities in different compartments of Ranbir Basmati

Compartments	Total number of bacteria	Ammonia production	IAA production	Phosphate solubilization	Cellulase	Catalase	Amylase	Siderophore	Protease
Rhizosphere	6	5	6	1	4	6	4	6	5
Rhizoplane	7	7	7	2	4	6	1	7	4
Endorhizosphere	5	5	5	2	3	5	2	5	4

Table 5: Numbers of bacteria exhibiting various plant growth promoting activities in different compartments of Basmati-129

Compartments	Total number of bacteria	Ammonia production	IAA production	Phosphate solubilization	Cellulase	Catalase	Amylase	Siderophore	Protease
Rhizosphere	3	3	3	2	3	3	3	3	2
Rhizoplane	5	5	5	3	5	5	5	5	4
Endorhizosphere	5	5	5	1	4	4	4	5	4