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Original article

Growth enhancement and changes in bacterial microbiome of cucumber plants exhibited by biopriming with some native bacteria

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ABSTRACT

This study investigated the impact of a mixture of six endophytic bacterial strains isolated from cucumber plants on the growth and microbiome diversity of six cucumber traditional varieties and hybrids. Six bacterial species were isolated and identified by 16 s rRNA sequencing. All the bacteria showed plant growth promoting traits. *Bacillus tequilensis* showed 80 % inhibition of the mycelia growth of *Fusarium oxysporum* f.sp. *cucumarinum* (Foc). Mixed culture of all the bacteria was prepared and applied back to the varieties and hybrids of cucumber plants through seed soaking. Plant growth characteristics indicated that the treated plants showed increased plant growth in terms of plant height, number of leaves, vine length, male:female flower ratio, number of fruits and fruit length. Bacteria treated plants of hybrid HiVeg Chitra recorded 19 cm increase in vine length compared to control plants. The matataxonomic analysis of leaf samples by Illumina sequencing highlighted a diverse bacterial community shift in treated plants, with significant increases in genera like *Bacillus* and *Staphylococcus*. The core microbiome analysis identified key genera such as *Bacillus, Staphylococcus, Sphingomonas, Methylobacterium*, etc that could be pivotal in plant growth promotion. *Bacillus* and *Staphylococcus* showed increased abundance in treated varieties, correlating with the observed in plant growth parameters thus indicating their role in growth promotion of cucumber plants. Endophytic bacterial species identified from cucumber plants when re-applied by seed soaking, they promote the plant growth by modulating the microbiome. The bacterial species identified in the study could be potential candidates as microbial bioinputs for cucumber cultivation.

1. Introduction

Endophytes are microorganisms that live within the tissues of plants and are found to play diverse functional roles in plant ecophysiology (Martínez-Rodríguez et al., 2014). These roles include enhancing plant growth, improving nutrient uptake, increasing tolerance to abiotic stresses, and protecting against pathogens (Wu et al., 2021). These microorganisms colonize the rhizosphere and the endophytic compartment, contributing to various aspects of plant development, fitness, and diversification (Lundberg et al., 2012; Hardoim et al., 2015). They have also been shown to enhance the resistance of host plants against various pathogens, including Foc which causes bacterial wilt in cucurbits (Zhou et al., 2023; Rojas et al., 2015; Wu et al., 2021). The characterization of endophytes and their functions can be achieved through advanced multi-omics methods, which involve integrating various omics technologies such as matataxonomics, transcriptomics, proteomics, and metabolomics (Kaul et al., 2016).

Matagenomics has emerged as a powerful tool for characterizing bacterial endophytes and understanding their interactions with host plants. Matataxonomic analysis allows for the study of the entire microbial community within a plant, providing insights into the diversity and functional roles of endophytes (Hardoim et al., 2015). By analyzing the genetic material present in the plant's microbiome, researchers can identify specific bacterial species and their potential contributions to plant growth promotion and disease resistance (Kaul et al., 2016). Matataxonomics can also reveal the genes and metabolic pathways involved in the establishment of endophytism and the production of plant growth-enhancing compounds (Dudeja et al., 2021).

The development of a bacterial mixture for plant growth and disease resistance against pathogens relies on understanding the function of bacterial endophytes and the importance of their characterization. Characterizing bacterial endophytes is crucial for their effective utilization as a bacterial mixture for plant growth promotion. By identifying and studying these endophytes, researchers can determine their plant

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growth-promoting (PGP) and biocontrol attributes, such as their ability to fix nitrogen, produce auxins, and solubilize phosphates (Raimi & Adeleke, 2023). This characterization allows for the selection of specific endophytes that are most effective against Foc and other plant pathogens. Additionally, understanding the shape and roles of the bacterial microbiota of plants provides insights into the mutualistic relationships between plants and endophytic bacteria, further highlighting the importance of their characterization (Bulgarelli et al., 2013; Hewedy et al., 2020).

Research has shown that certain Zinc nanoparticles, plant growth-promoting rhizosphere bacteria (Seleiman et al., 2023), and bacterial endophytes, such as *Bacillus subtilis* isolated from Prosopis glandulosa rhizosphere (Abdelmoteleb et al., 2023) and *Bacillus tequilensis*, have biocontrol potential towards biotic stress-causing agents like *Erysiphe heraclei* DC, *Fusarium* species, including Foc (Baard et al., 2023; Ahmed et al., 2021). *Trichoderma* species are also well-known biocontrol agents for soil-borne pathogens including *Fusarium* species (Hewedy et al., 2020). These endophytes can inhibit the growth of *Fusarium* and protect plants from root rot diseases (Amiri & Tibuhwa, 2021). Furthermore, investigations have shown that endophytic fungal extracts and synthesis of agriculture nanoparticles derived from plants such as cashew trees and *Avena fatua* L. (wild oat) exhibit antibiotic properties against *Fusarium oxysporum* (Qureshi et al., 2023; Elbrense et al., 2021). Additionally, in salt-stressed cucumbers, repeated administration of antioxidants improves ion imbalance and enhances antioxidant systems (Seleiman et al., 2020). These findings emphasize the potential of endophytic bacteria and fungi in developing bacterial mixtures for improved plant development and disease resistance.

With this background, the current study aimed at exploring the endophytic bacterial species from cucumber plants, applying them back to the plants after characterization, and observing the growth parameters and microbiome changes in treated plants.

2. Methodology

2.1. Sample collection, preparation, and endophytic bacterial isolation

The seeds of six distinct cucumber varieties were gathered from local farmers, including two hybrids (Seminis Malini and Hyveg Chitra) and four traditional varieties (Agathiyar Heirloom, Spiny, Fruit, and Round cucumber). All types of seeds were planted in polybags and grown in a net house for 45 days for the collection of samples. A handful of each variety of seeds were taken and washed thoroughly in flowing water. Two grams of leaves and seeds were collected and disinfected for 1 min using 70 % alcohol, 2 min of 4 % sodium hypochlorite, 30 sec of 70 % ethanol, and final rinsing with sterile water. The seeds and leaves were ground in a disinfected grinder with injection water. The tissue extract was then incubated at 28 °C for three h to permit the complete discharge of endophytic microorganisms from the surrounding tissue. The extract of tissue was diluted with injection water and plated on nutrient agar and enrichment medium (HiMedia) to isolate endophytic bacteria. The plates were incubated for up to 48 h at 35 °C and colonies were chosen every 16 h. The colonies were chosen based on their morphology (color, size, and shape).

2.2. Molecular identification of bacteria

Identification of endophytic bacterial analysis was done by using the colony polymerase chain reaction (PCR) method. Single colonies were suspended individually in 30 µl of tris-ethylene diamine tetra acetic acid (TE) buffer and mixed well. After boiling at 90 °C for 10 min, cultures were centrifuged at 4 °C for 10 min at 10,000 rpm. The supernatant was gathered and utilized as a template DNA for amplifying the NB16sF (5′-AGAGTTTGATCATGGCTCAG – 3′) and NB16sR (5′- ACGGCTACCTTGTTACGAC –3′) universal primers. The 20 µl reaction mixture contained 2 µl of template DNA, 1 µl each of forward and reverse primers, 10 µl of 1X master mix (Amplicon), 6 µl of nuclease-free H_2O. The amplification was done in a DNA thermal cycler (T100, BioRad, Singapore). The PCR conditions were: initial denaturation for 10 min at 94 °C, 30 cycles each consisting of denaturation for 1 min at 94 °C, primer annealing for 1 min at 49 °C, extension for 1 min at 72 °C, and final extension for 7 min at 72 °C (Nithya and Babu, 2017). The amplified products were sequenced at BioKart India Pvt Ltd., Bangalore, India. The identification was done using BLAST in NCBI. Each isolate's 16S rDNA genome sequence was deposited in the NCBI GENBANK repository under the database accession codes OR489015, OR489016, OR489017, OR489018, OR489019, and OR489020.

2.3. Antifungal activity and plant growth promotion tests

The antifungal property of bacterial isolates was tested against the test pathogen Foc using a dual culture technique on potato dextrose agar medium. The measurement of the zone of inhibition's diameter was done at 48-h intervals over 7 days. All the antagonistic endophytes underwent plant growth-promoting tests including assessments of phosphate solubility, production of siderophore, indole-3-acetic acid, ammonia, and nitrogen fixation. The compatibility of bacterial endophytes was assessed by using a cross-streak assay, and biochemical assays were conducted using the KB001 TM HiIMViC Biochemical Test Kit (Himedia) (K et al., 2021).

2.4. Preparation of bacterial inoculum and seed treatment

The bacterial liquid mixture was prepared using the six antagonistic bacterial endophytes (*Bacillus tequilensis, Bacillus licheniformis, Nesterenkonia aethiopica, Nesterenkonia suensis, staphylococcus warneri, and Microbacterium arborescens)* with an equal ratio. Seed treatment was done using the seed soaking method. Twenty seeds of each variety were taken and soaked in 10 ml of liquid mixture for 6 h. After soaking, seeds were kept for drying on sterile filter paper under a laminar airflow chamber for 15 min and were sown in experimental polybags in the greenhouse (Amutha, 2017).

2.5. Experimental setup and plant growth

Cucumber plants were grown in plastic bags (24 × 24 cm), with each bag containing a 20 kg potting mix consisting of 14.7 kg of garden soil (sandy loam texture), 5 kg of worm manure, 0.0625 kg each of Nimba cake, Ricinus cake, and Pongamia cake, 0.0200 kg of diammonium phosphate, and 0.0150 kg of potash. Both the treated and untreated seeds were sown separately in a potting mix, two seeds in each bag, and cultivated in the net house. The experimental design was a randomized block design with three replications per treatment per variety. Each replication is a grow bag with two plants each. The soil had a pH of 6.5, a coarse texture, and was deep brown. The plants have been exposed to a natural photoperiod of 12 h of continuous sunlight followed by 12 h of darkness. The temperature was kept between 25 and 27 °C during the day and 18 and 20 °C at night. The use of sensors and sprinklers ensured that relative humidity levels were maintained at 60–70 % during the day and 70–80 % at night. Data was recorded on growth and physical parameters. The leaves of both control and treatment plants were collected during the vegetative stage (40 days after sowing) of plant growth and afterward subjected to matataxonomic analysis.

2.6. Nucleic acid extraction and matataxonomic analysis

The metataxonomic study was conducted by targeting v3-v4 (16 s rRNA) regions of the genome of the samples, by employing the default parameters (Biokart India Pvt. Ltd., Bangalore, India). The DNA of the genome was isolated using a Qiagen DNeasy Plant Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. Following that, the nucleic acid's purity and quantity were assessed using a Nanodrop, a

spectrophotometer (Thermo Scientific, USA), and gel electrophoresis, respectively. The libraries were constructed using Illumina adapters which were barcoded and subsequently filtered using Ampure bead particles. The sequencing was performed on the Illumina Miseq platform (Biokart India Pvt Ltd.). The raw sequences were submitted to the NCBI Sequence Read Archive (SRA) database and assigned the accession numbers listed below. 1. Leaf control (SAMN38113883, SAMN38113884, SAMN38113885, SAMN38113886, SAMN38113887, SAMN38113888); 2. Leaf treated (SAMN38113889, SAMN38113890, SAMN38113891, SAMN38113892, SAMN38113893, SAMN38113894).

2.7. Bioinformatic analysis

The initial assessment of quality of the 16S rRNA sequence data was performed with FastQC and MultiQC tools. Subsequently, TrimGalore tool was employed to remove adapter sequences which are non-bacterial origin. Sequence readings with low quality were eliminated employing a default threshold value of less than 20. The processed reads underwent additional steps, including as merging of pair-ends, elimination of chimeras, and determination of abundance of operational taxonomic units (OTUs). These steps were performed using the QIIME workflow, which also included estimation correction. The implemented procedure facilitated precise research at the taxonomic level of the bacterial genera with a high degree of accuracy. The database utilized in this study was the National Centre for Biotechnology Information.

Alpha diversity was assessed by making use of Shannon indexing, which provides insights into both the quantity and distribution of bacterial species within a given sample. Additionally, the Chao1 estimate, which approximates the total species quantity was employed in an alpha diversity analyzer tool. The Chao1 approach is used to assess richness of every bacterial genera by estimating the number of unusual organisms that may have gone unnoticed due to poor sampling. The Shannon scale is used to characterize the observed variation within a given population. The whisker plots demonstrate the range of diversity values recorded within a population, excluding any unusual values represented as individual data points, and are based on the Chao1 and Shannon indices with a significant threshold $< P\ 0.05$ (Navya and Babu, 2023).

The principle coordinate analysis (PCoA) method was employed to represent the beta variance among samples (leaf tissue of cucumber traditional varieties and hybrids) in a two-dimensional display, where every point refers to the whole biome inside a specific sample. The percentage of variance detected between the samples is shown by each axis. The horizontal axis corresponds to the degree of deviation with the highest magnitude, while the vertical axis corresponds to the degree of deviation with the second highest magnitude. Permutational ANOVA (PERMANOVA) can be used to assess the statistical impact of the grouping style in ordination panels.

Heat maps were constructed through the heat map algorithm in R and Quantitative Insights into Microbial Ecology software packages to elucidate the overall abundance, frequency, and functional characteristics of microbial variation. A visual representation of a data structure is presented using a histogram, wherein an application of color serves to provide a concise overview of numerical disparities.

3. Results

The samples used for the metataxonomic sequencing are labelled as follows: CT1L, CT2L, CT3L, and CTS1L- leaf samples from untreated plants of traditional varieties Agathiyar heirloom, Fruit variety, Spiny variety, and Round variety respectively. TT1L, TT2L, TT3L, and TTS1L are the treated counterparts of the above traditional varieties. CH1L, CH2L- leaf samples from untreated parts of modern hybrids Seminis Malini and HiVeg Chitra, respectively. TH1L and TH2L are the treated counterparts of the above hybrids. The first letter of the code C or T refers to control or treated; second letter T or H refers to traditional variety or hybrid; the number in the code indicates first, second or third variety / hybrid.

3.1. Morphological characteristics, biochemical tests, and bacterial mixture

Morphological characteristics tests revealed that all six bacteria were gram + ve, rod-shaped, and white except the *Staphylococcus warneri* which is spherical in shape and pale orange in color (Table 1, A). Plant growth promotion trait tests revealed that all six bacteria are positive in the secretion of indole-acetic acid, ammonia, and fixation of nitrogen. *Nesterenkonia suensis* and *Microbacterium arborescens* were negative in siderophores and phosphate solubility production. The antagonistic activity test revealed that the *Bacillus tequilas, Bacillus licheniformis*, and *Nesterenkonia aethiopica* showed growth inhibition of Foc at 80 %, 20 %, and 60 % respectively (Table 1, B). Biochemical tests revealed that all six bacteria are positive in utilizing glucose, lactose, mannose, and sucrose and all are negative towards voges Proskauer, adonitol, and arabinose (Table 1, C). The bacterial liquid mixture was developed using all six endophytic bacteria viz., *Bacillus tequilensis, Bacillus licheniformis*, *Nesterenkonia aethiopica, Nesterenkonia suensis, staphylococcus warneri, and Microbacterium arborescens.*

3.2. Plant growth parameters

Both control and treated plant physical parameters were collected. All the plant physical parameters were improved in plants treated with liquid mixture (Table 2). The plant's vegetative parts like vine length, root length, leaf count, and chlorophyll content have improved in all treated varieties compared with fruiting. Statistical analysis was performed using JMP software (Fig. 1).

The highest plant physical parameters recorded in the control hybrid variety HiVeg Chitra (CH2L); seed germination percentage (100 %), leaf count (72 number), vine length (144 cm), root length (17.1 cm), male/female ratio (48:18), fruit count (16 number), and fruit length (18 cm). The highest plant physical parameters recorded in the control traditional variety Agathiyar heirloom (CT1L); seed germination percentage (90 %), leaf count (60 number), vine length (120 cm), root length (12.6 cm), male/female ratio (39:10), fruit count (7 number), and fruit length (14 cm).

The highest plant physical parameters recorded in the treated hybrid variety HiVeg Chitra (TH2L); seed germination percentage (100 %), leaf count (78 number), vine length (163 cm), root length (20.6 cm), male/female ratio (60:22), fruit count (20 number), and fruit length (20 cm). The highest plant physical parameters recorded in the treated traditional variety Agathiyar heirloom (TT1L); seed germination percentage (100 %), leaf count (68 number), vine length (146 cm), root length (15.1 cm), male/female ratio (41:13), fruit count (11 number), and fruit length (17 cm). The highest SPAD values in control 50 nmol/cm and treated 52 nmol/cm were observed in the control traditional and treated traditional varieties, Agathiyar heirloom (CT1L, TT1L) and Spiny variety (CT3L, TT3L). All the plant physical parameters were found to be lowest in the control and treated variety of Round variety (CTS1L, TTS1L).

3.3. Alpha and beta diversity of an endophytic bacteria

By employing this methodology, it is possible to evaluate the amount of diversity present within a given sample or group. Alpha diversity can be quantified in terms of the total richness of species, their evenness, or metrics that combine both. This study targeted to understand the prevalence of bacterial colonies seen in endophytes after treatment with mass-multiplied endophytic bacteria in traditional and hybrid varieties of cucumber plants. The statistical analysis of the metataxonomic data conducted using Shannon and Chao1 P less than 0.05 tests showed significant variation in the diversity of microbes between traditional varieties and modern hybrids. The whisker bar depicts the range of major and minor values for alpha deviations in a group, after removing

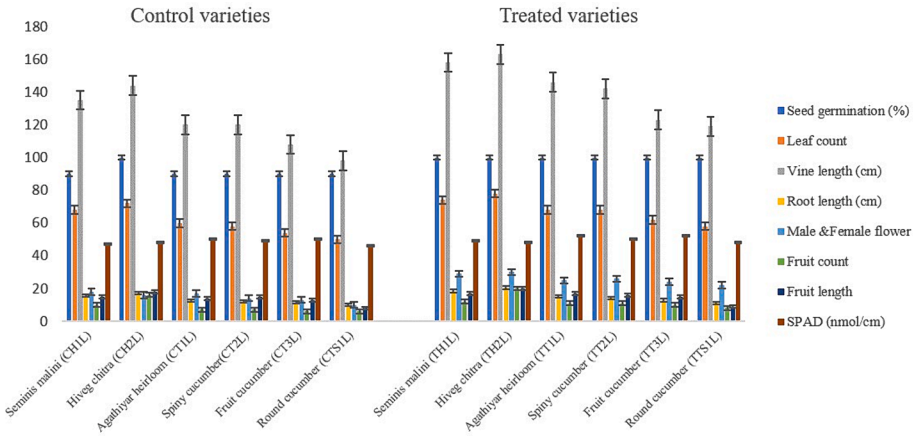

Fig. 1. Statistical analysis of plant growth and physiological parameters recorded in both cucumber plants treated with bacterial mixture and untreated control.

extremes that appear as single points.

Chao1 and Shannon's indices found that the overall bacterial variations and richness are greater in traditional varieties treated with the bacterial mixture. The bacterial diversity was observed highest in the treated traditional plant TT1L compared with its untreated control. Whereas the bacterial diversity in untreated and treated traditional varieties (CT2l, CT3L, CTS1L) (TT2L, TT3L, and TTS1L) was found to be similar (Fig. 2 A, B). The bacterial abundance was higher in traditional varieties TT1l, and TTS1L treated with bacterial mixture compared to their respective untreated controls. Bacterial richness was even in traditional varieties TT2L, and TT3L compared with control (Fig. 2 C, D). In hybrids, the overall endophytic bacterial diversity and richness are higher in treated hybrid plants compared to their untreated counterparts. The bacterial diversity and richness are highest in treated hybrid TH1L, and TH2L (Fig. 2 A, B, C, D).

A comparison of traditional and hybrid varieties indicates that the variation is greater in traditional plants than in hybrid plants, both in the case of untreated control plants and plants treated with bacterial mixtures.

Beta diversity of each variety and hybrid displayed a unique abundance and diversity of OTUs. The matrices were depicted graphically in both two-dimensional (2D) (Fig. 2 E) and three-dimensional (3D) (Fig. 2 F) plots utilizing the ordination-based technique known as Principle Coordinate Analysis (PCoA). Each point on the plot corresponded to the entire microbiota composition of an individual sample.

Among the traditional cucumber varieties treated with a bacteria mixture, the endophytic bacterial communities are a little closer to each other and are far from the untreated control plants. The treated traditional varieties TT3L, TTS1L, and the control traditional variety CT3L formed a group. The remaining treated traditional varieties TT1L, TT2L, and control traditional varieties CT2L, CT1L, and CTS1L are far from each other. In the matter of hybrids, the endophytic bacterial colonies of treated hybrid TH1L, and TH2L formed a group along with the control CH1L. The control hybrid CH2L alone was found to be separate (Fig. 2 E).

3.4. Core microbiome of control and treated varieties

The core microbiome encompasses the endophytic bacterial genera that are common among all the samples under evaluation. Microbiome clusters were created to analyze the endophytic bacteria in both control and treated cucumber varieties and hybrids, with a focus on gene representation. *Staphylococcus* showed the highest abundance with 1 % whereas *Bacillus, Clostridium, Sphingomonas, Methylobacterium, Pseudomonas, Streptomyces, Acinetobacter, Lactobacillus, Acetobacter,* and *Brevundimonas* showed the abundance 0.9 %. contributing to *Aureimonas, Ralstonia,* and *Corynebacterium* recorded at 0.8 % followed by *Prevotella* and *Streptococcus* (0.6 % and 0.5 % respectively) (Fig. 3).

3.5. Co-networking of endophytic bacteria in control and treated varieties

A co-network analysis was done to get insights into the interactions occurring among bacterial species. The impact of the microbiome on the functioning of the plant may be largely determined by the species with the greatest number of networking partner species. Many taxa were identified with the use of microbial network analysis, which also predicted significant species and their relationships. *Aureimonas, Methylobacterium, Sphingomonas, Bacillus, Clostridium,* and *Fusobacterium* were identified as taxa that had a significant presence in both the treated and control plants microbiomes, suggesting their potential importance as essential contributors to the microbial community of cucumber plants (Fig. 4).

3.6. Major bacterial genera

Based on the operational taxonomic units count the top ten bacterial taxa in control and treated plants of traditional and hybrid varieties are presented in Fig. 5. *Aureimonas, Sphingomonas, Methylo bacterium, Bacillus, Pseudomonas*, and *Clostridium* were present in both control and treated traditional varieties (Fig. 5 C-F and I-L). *Aureimonas, Methylobacterium, Sphingomonas,* and *Clostridium* were present in both control and treated hybrid varieties (Fig. 5 A, B and G, H). *Fusobacterium, Bacteroides, Lactobacillus, Bacillus,* and *Staphylococcus* were present only in traditional varieties. Control hybrid varieties did not show any unique bacterial genera compared with control traditional varieties (Fig. 5 A-F).

Brevundimonas, Acetobacter, Bacillus, and *Pseudomonas* were present only in traditional varieties. Treated hybrid varieties did not show any unique bacterial genera compared with treated traditional varieties (Fig. 5 G-L). The top 10 % bacterial genera are noted which are listed below and these could be significant players in the development and productivity of cucumber plants. *Bacillus*, *Aureimonas, Clostridium, Fusobacterium, Sphingomonas,* and *Methylobacterium* were present in both control and treated plants. *Fusobacterium, Bacteroides*, *Streptomyces, Pseudomonas, Lactobacillus, and Staphylococcus* were present only in control plants whereas *Brevundimonas* alone was found in treated plants (Fig. 5 A-L).

4. Discussion

Over the past few decades, the bacterial microbiota has been manipulated in crops to enhance crop health (Kwak et al., 2018). This has subsequently led to increased exploration of microbiota associated with crops. Bacterial endophytes, commonly found within plants, have been the focus of numerous studies. These investigations consistently

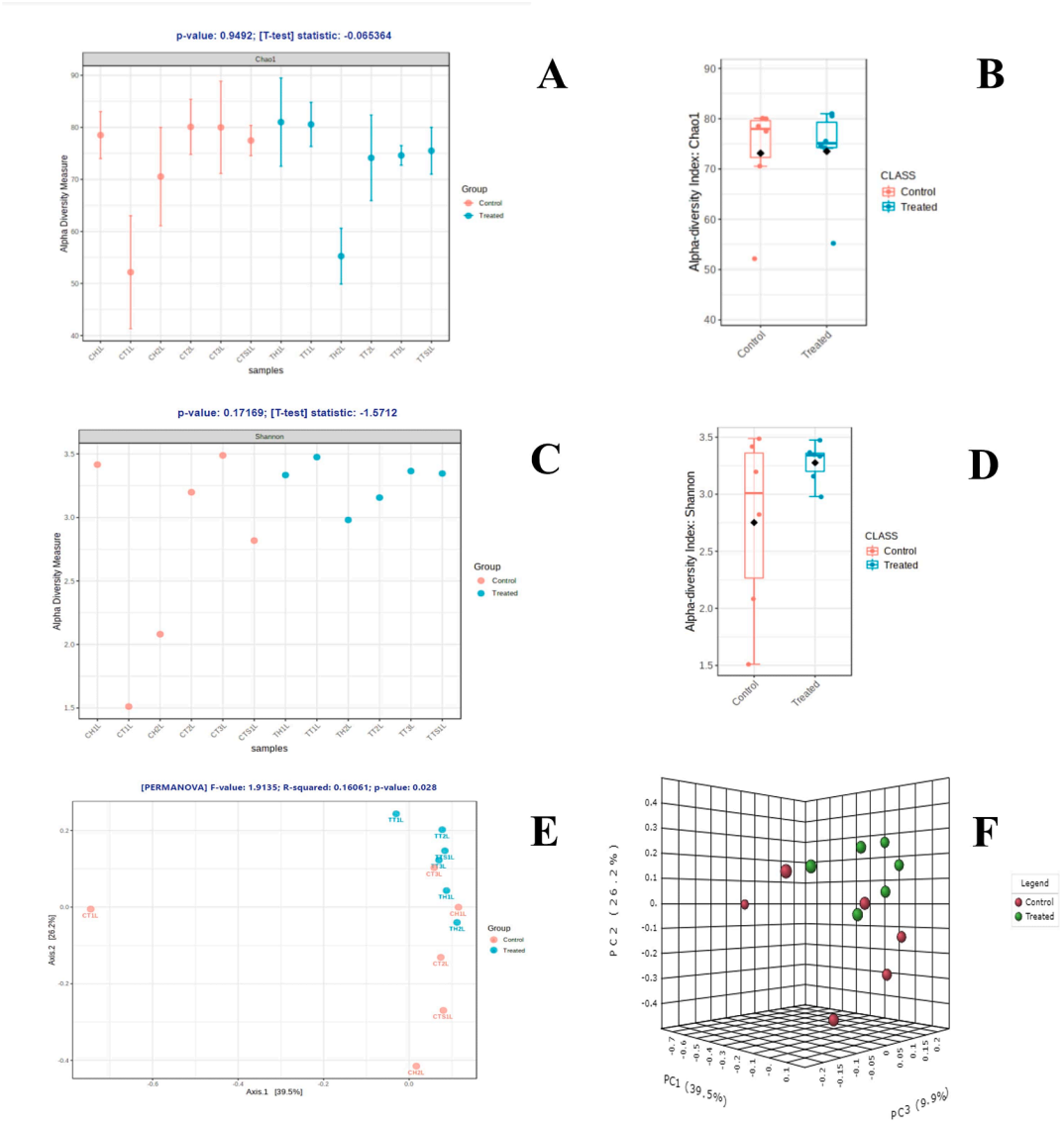

Fig. 2. Alpha diversity and PCoA of endophytic bacteria in leaves of cucumber plants. Fig. A and B depict the Chao 1 and box plot; Fig. C and D depict the Shannon index and box plot; Fig: E depicts the cluster formation of endophytic bacteria in control and treated varieties; Fig: F depicts a 3D representation. Alpha diversity can be quantified in terms of the total richness of species, their evenness, or metrics that combine both. According to the Chao1 and Shannon indices, samples show varying levels of bacterial richness and diversity on the X and Y, respectively. The outliers from the following diversity are considered to have less diversity. The statistical significance of the . (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.) Source-based grouping (leaves of treated varieties = blue color; leaves of untreated control varieties = peach color) is determined using the Kruskal-Wallace test, which has a significance level of $P < 0.05$. Principle coordinate analysis (PCoA) based 2D and 3D ordination graphs are used to illustrate the results. This was performed based on the non-phylogenetic Bray-Curtis distance method. Permutational multivariate analysis of variance is used to determine the corresponding statistical significance (PERMANOVA). The color of the samples on the PCoA plots is based on the image legends

demonstrate the potential of endophytes to stimulate plant development and improve host plants' innate tolerance against various stressors (Tian et al., 2022). This study examined the plant growth promotion in hybrid and traditional cucumber varieties treated with bacterial mixtures containing endophytic bacteria which promotes plant growth. Further, the study was extended to analyze the alterations in the endophytic bacterial diversity present in hybrid and traditional cucumber plants that were treated with the bacterial mixture, comparing them with untreated control plants.

About six bacterial species were isolated from leaves and seeds of cucumber and identified as *Bacillus tequilensis, Bacillus licheniformis, Staphylococcus warneri, Nesterenkonia aethiopica, Nesterenkonia suensis,* and *Microbacterium arborescens*. The results of the tests indicate that all the bacterial isolates had plant growth-promoting abilities and antagonistic activity with cucumber wilt pathogen Foc.

B. tequilensis is a species of bacteria that has been studied for its potential applications in biological control and plant protection. *B. tequilensis* has been found to exhibit antifungal activity against various fungal pathogens, including *Magnaporthe oryzae* and *Fusarium* species. The mechanisms by which *B. tequilensis* inhibits fungal growth include the production of antifungal metabolites, lipopeptides, and other bioactive compounds. These compounds can disrupt fungal cell membranes, inhibit spore germination, and interfere with fungal growth (Ali et al., 2020). According to Wang et al. (2022), *B. tequilensis* SX31, improved cucumber plant growth by dissolving the inorganic and organic phosphorus and by producing auxin.

B. licheniformis is beneficial to increase the total nitrogen content in cucumber. The combination inoculation of *B. licheniformis* and *B. subtilis* enhanced cucumber growth in seedlings under salt stress, increased the amount of nutrients, and mimicked enzyme activity. (Qi et al., 2021).

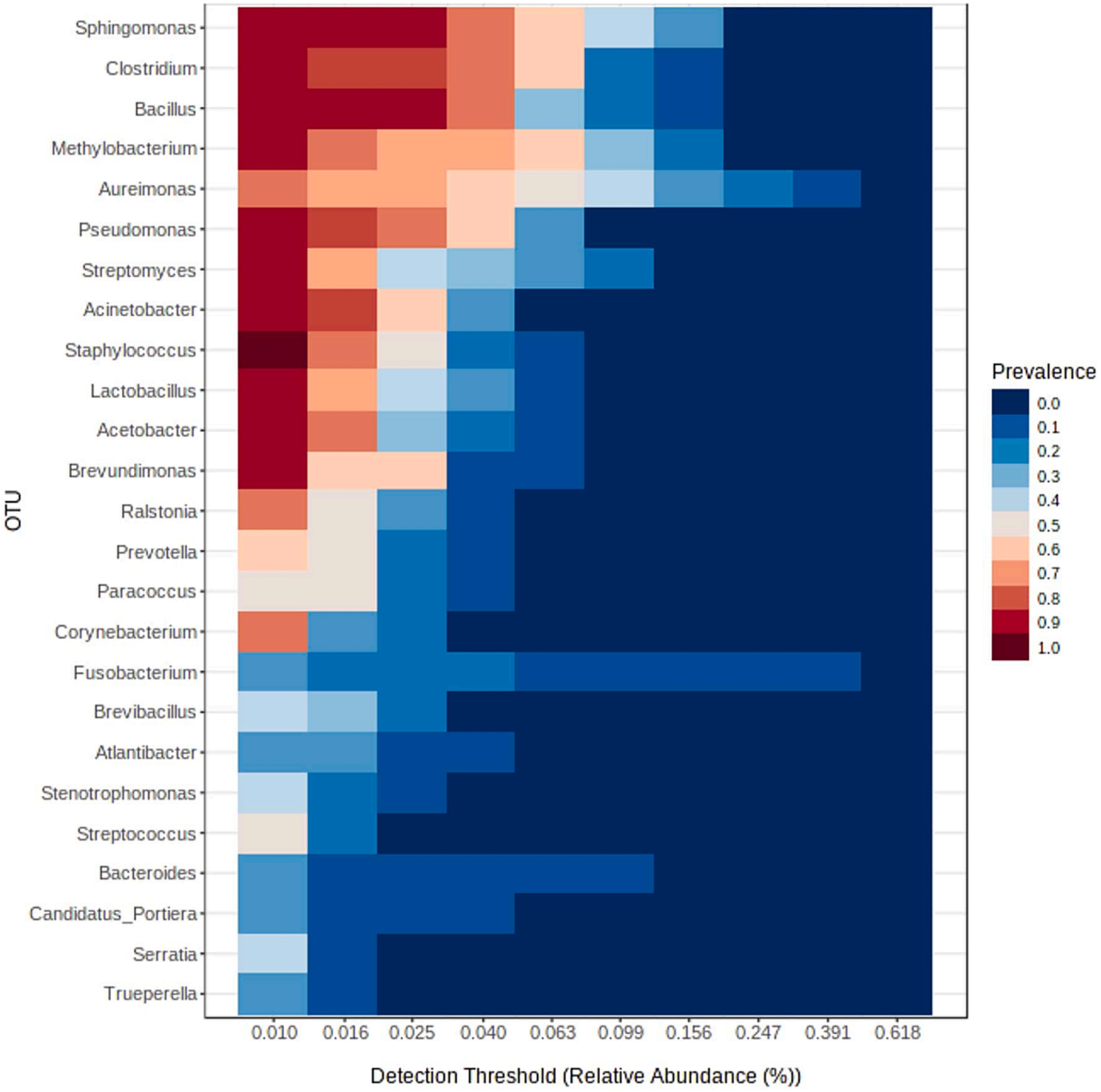

Fig. 3. Heat map of the core microbiome bacteria in leaves of cucumber plants. The heat map is constructed based on the relative abundance of OTUs and colored based on the predominance. Bright red exhibits a 1.0 % for predominance but bright blue displays 0.1 %. The healthy core microbiome represents 11 different bacterial genera. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

According to Goswami et al. (2014), *B. licheniformis* strain showed the most notable PGP traits *in vitro* and *in vivo* pot culture experiments. This strain enhanced the biomass and plant length in *Arachis hypogea*, when an additional 50 mM NaCl was supplemented in the soil.

Agarwal et al. (2020) discovered that *S. warneri* obtained from *Gnetum gnemon* could release different siderophores with a strong affinity for Fe3+, successfully combating the spread of *Ralstonia solanacearum* in the root zone. According to Ahangir et al. (2019), *S. warneri* is a viable option for developing an economical supply of phosphate fertilizers that improve plant growth while also helping to utilize the existing soil phosphate. *S. warneri* is a possible plant growth-enhancing rhizobacterium of cotton, swainsonpea, cashew, maize, and rice with nitrogen-fixing, saline-tolerance, and antagonism properties (Dang and Do, 2018).

M. arborescens has been shown to produce different polysaccharides that have the property of cementing soil particles together (Bhagat et al., 2021). A chemotaxonomic study performed by Godinho and Bhosle (2013) found that the isolated organism comprised peptidoglycans type B1 with diamino acid L-lysine, galactose, and rhamnose, as cellular wall sugars, and related completely to the genera *Microbacterium*. Surprisingly, this isolate was discovered to generate substantial amounts of exopolysaccharide, which may aid in its adhesion and viability in this often-stressed environment. *N. aethiopica* and *N. suensis* are halophiles and alkaliphilic bacteria (Delgado et al., 2006; Govender et al., 2013), Gómez-Acata et al. (2021) reported that members of *Nesterenkonia* can produce siderophores, which are already known to produce halophilic proteases and xylanases.

In this current study for the first time, we have found that *N. aethiopica* showed strong antagonistic activity (60 %) towards Foc. Alongside both of the *Nesterenkonia* species showed positive results in

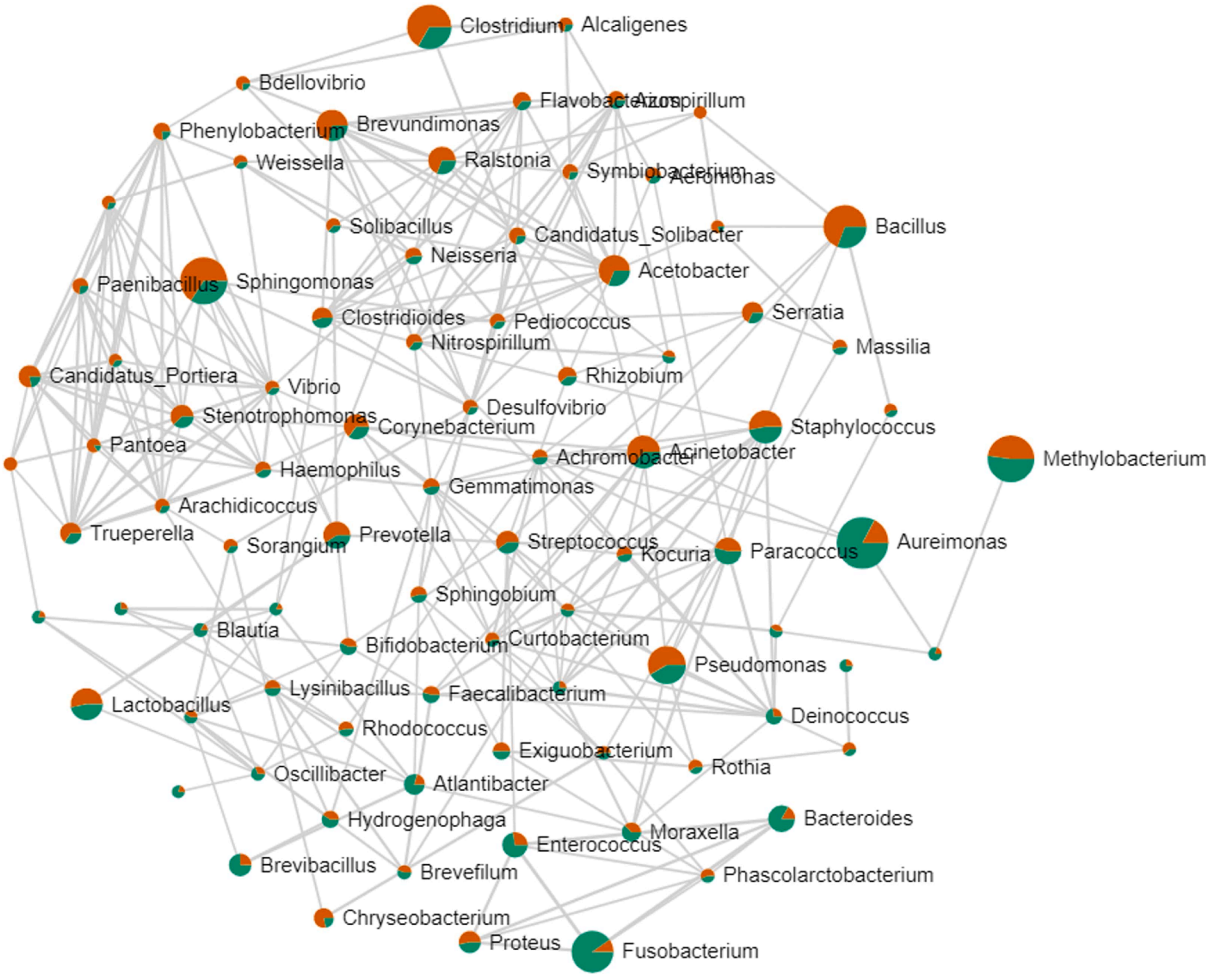

Fig. 4. Network analysis of endophytic bacteria in cucumber plants treated with endophytic bacterial mixture. The various colors denote the different varieties of cucumbers, while the size of the nodes indicates the abundance of OTUs. The orange color represents the treated varieties and the Green represents the untreated control varieties. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the *in vitro* PGPR tests like siderophore, nitrogen, indole acetic acid, and ammonia production. When applied as a mixture, *B. tequilensis* and *S. warneri* would have contributed to the plant growth in cucumber due to their phosphate solubilizing ability and production of auxin. *B. licheniformis* would have played a major role in increasing the biomass and plant height. *M. arborescens* due to its polysaccradies have improved the soil properties thus enhancing better root growth and nutrient absorption in the bactereial mixture treated plants, which was evident as better above soil plant growth.

Furthermore, this study analyzed the bacterial diversity shifts and functional metabolism in plant microbiomes. In total, endophytic bacteria belonging to 15 genera, including *Sphingomonas, Clostridium, Bacillus, Methylobacterium, Aurimonas, Pseudomonas, Streptomyces, Acinetobacter, Staphylococcus, Lactobacillus, Acetobacter, Brevundimonas, Ralstonia, Prevotella,* and *Corynebacterium* were detected in core microbiome. The abundance of bacterial genera *Sphingomonas, Methylobacterium, Streptomyces, Aurimonas, Staphylococcus, Pseudomonas* and *Clostridium* was common in both treated and control hybrid and traditional varieties. Interestingly the genera *Bacillus* and *Staphylococcus* showed an abundance in treated hybrid and traditional varieties compared with untreated control varieties, which indicated that these two bacterial genera played a prime role in plant growth promotion.

In our previous study, we have observed *Lactobacillus* and *Prevotella* as endophytic in the traditional and hybrid varieties of cucumber under greenhouse conditions (Navya and Babu, 2023). Zhou et al. (2022) reported the presence of *Pseudomonas* and *Streptomyces* in the rhizosphere of specific greenhouse cucumber varieties. Typically, plant roots secrete specific substances that facilitate the multiplication of specific species of bacteria or fungi in the root zone. This, in turn, leads to the establishment of the fundamental microflora within the community of rhizosphere microorganisms and enters inside the plant (Doornbos et al., 2012; Wen et al., 2020). Thus, *Bacillus* and *Staphylococcus* observed as abundant in metataxanomic data in treated varieties could likely belong to the isolates applied through the bacterial mixture that are attracted by the root exudates of the germinated cucumber seedlings. All the endophytic bacterial genera in the core microbiome could be key players but, since the abundance of bacterial diversity shift was observed in *Bacillus* and *Staphylococcus,* these two could be the prime players for cucumber plant growth. *Staphylococcus* and *Bacillus* are known as plant growth-enhancing bacteria (Thanh and Tram, 2018; Wang et al., 2022) and biocontrol agents. *Bacillus* is a capable biocontrol agent for the Foc which can control the wilt disease in cucumber (Ali et al., 2020; Khan et al., 2018). The entire work was majorly focused on the endophytic bacterial diversity shifts after the application of a bacterial mixture containing the mass multiplied bacterial isolates.

Although the metataxonomic data reveals at the genus level and could not be confirmed whether the observed *Bacillus* and *Staphylococcus* in the microbiome in treated plants are from the mixed culture applied, there is chance to presume that they are same, due to the observed plant growth parameters. These two bacteria in the mixed culture would have been a dominating to outnumber the number of cells of other bacteria in the mixture to occupy the endophytic environment in the cucumber

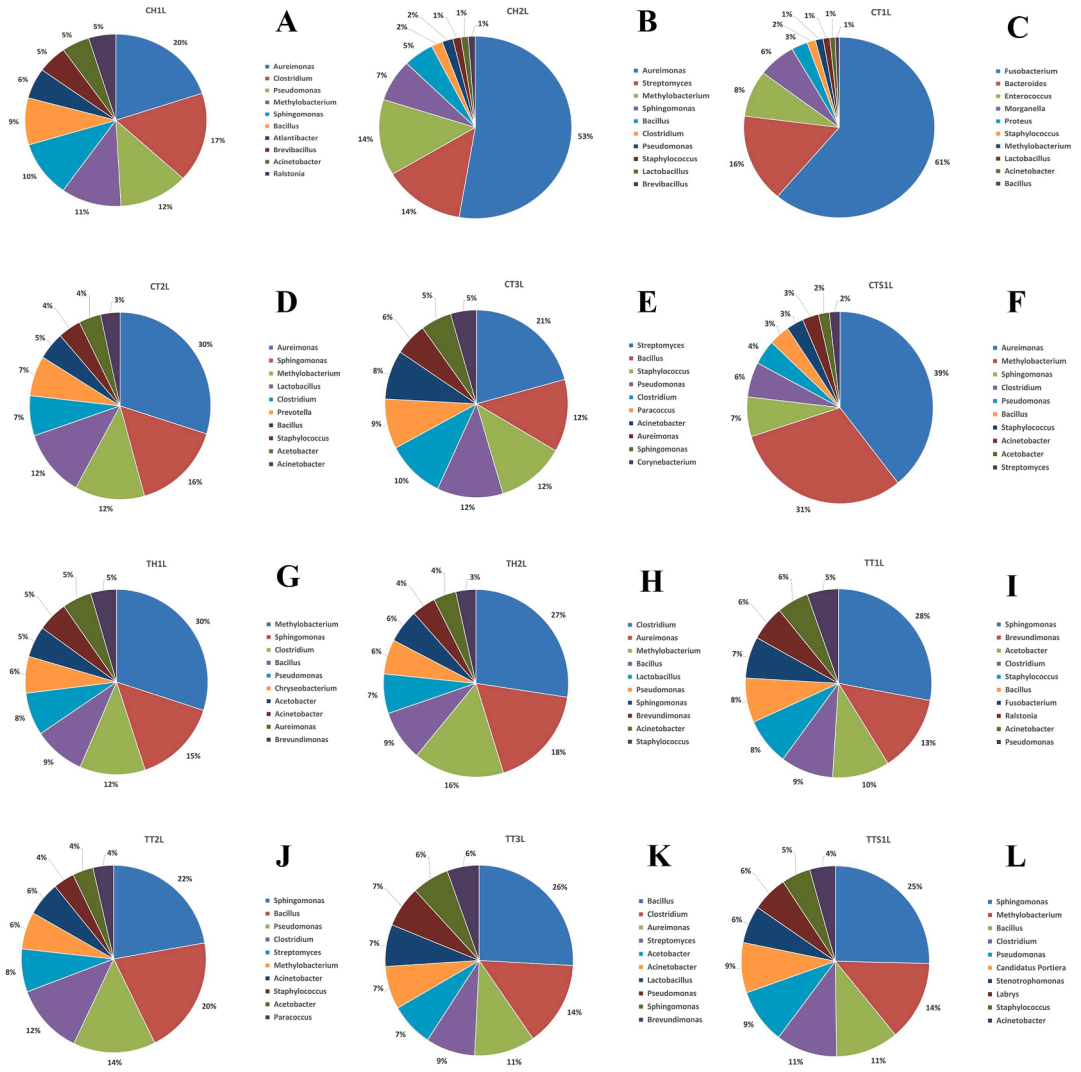

Fig. 5. Top ten abundant endophytic bacteria present in untreated and treated cucumber traditional varieties and hybrids. A – B. Untreated hybrids; C – F. Untreated traditional varieties; G – H. Treated hybrids; I – L. Treated traditional varieties.

plants by colonizing faster when the plants germinated after seed treatment. Nevertheless, the individual abilities as per the previous reports may not be enough to convincingly conclude the mechanism behind the growth promoting role of the mixed culture. Existence of these individual species in the mixed culture without competition and retaining the functions while applied to plant, are yet to be studied before developing this mixed culture as a microbial bio-input for cucumber cultivation.

CRediT authorship contribution statement

Navya Botlagunta: Investigation, Methodology, Writing – original draft. **Subramanian Babu:** Conceptualization, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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