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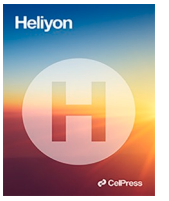

Comparative metataxonamic analyses of seeds and leaves of traditional varieties and hybrids of cucumber (*Cucumis sativus* L.) reveals distinct and core microbiome

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ABSTRACT

Profiling the endophytic microbiome of different tissues and varieties of agricultural crops can help to understand i) the tissue specific and varietal specific microbes associated with the plants ii) their potential role in growth, stress tolerance, disease resistance, and yield of the plants. Comparative microbiome profiling across various varieties and hybrids will also be useful to identify the plant's core microbiome. The main objective of the work is to profile and study the microbiome of traditional varieties in comparison with hybrids of cucumber, which would help to understand the microbiome structure in developing consortia to engineer the microbiome of modern hybrids, for useful phenotypes. Metataxonomic sequencing of bacteria and fungi using 16S rRNA gene and ITS regions respectively were carried out in seed and leaf samples of cucumber traditional varieties and modern hybrids. Among bacteria, *Prevotella, Bacteroides, Lactobacillus, Dialester,* and *Fecalibacterium,* and among fungal genera, *Pichia, Aspergillus, Phaeoisariopsis, Candida,* and *Malassezia* belonged to the core microbiome of cucumber. Modern hybrids were rich in antibiotic producing and toxic pollutant degrading bacteria. Many of the fungi and bacteria observed in the study are well known plant growth promoting microorganisms and play role in offering disease resistance. Some of the bacteria and fungi have beneficial roles in human gut thus revealing the dietary importance of cucumber. The microbes identified in the current study will be useful starting point to develop a consortia to engineer the cucumber microbiome for growth, yield and stress tolerance traits.

1. Introduction

Cucumber is known as a water vegetable that belongs to the Cucurbitaceae family with a lot of nutrients yet very low in calories. Cucumber is a widely cultivated and widely consumed fruit in the form of fresh, cooked, or pickled [1]. Since ancient times, the cucumber's therapeutic benefits have been documented. The therapeutic potential of the plant's various parts, including the leaf, fruit, and seed, has been investigated [2].

Plants and microbes have developed a close symbiotic interaction over a lengthy period of coevolution. These microbes reside outside (epiphytes) and inside (endophytes) entire plant tissues, carrying out crucial ecological duties [3]. For instance, several endophytes like bacteria and fungi serve crucial roles in the growth, stress tolerance and production of a variety of useful bioactive

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metabolites. When endophytes interact with their hosts, a variety of microorganisms can increase the plant's resistance by producing a variety of bioactive compounds [4]. Studies have shown that endophytic population, diversity and community structure vary between varieties of a crop plant [5].

The interaction between endophytes and plants is a growing topic in the field of plant research [6] but the difference in endophytic microbial diversity in traditional and hybrid verities is not yet explored deeply. Traditional varieties of crops are locally preserved germplasm which are not cultivated widely and repeatedly. Most of the traditional varieties of crops are known to have beneficial traits such as disease resistance, better abiotic stress tolerance and nutritious produce. The structure of the phytobiome can be changed by conventional agricultural techniques such as fertilizer, tillage, and chemical pest and pathogen management [7]. The compatibility of agricultural crops and the microbes they associate with may also vary as a result of the loss of genetic variety brought about by breeding programs and domestication, resulting in modifications to the phytobiome of crops in comparison to their wild varieties [8, 9]. As a result, it is possible for traditional varieties to have a variety of endophytes, including helpful species, that are absent in modern varieties and hybrids. Moreover, the endophytic microbiome may differ in different plant parts such as seed, root, stem, leaves, flowers and fruits.

Currently, a variety of works have been published on the incorporation of microorganisms in host plant seeds using the 16S rDNA clone library method [10], culture-dependent method [11,12], and the high-throughput sequencing (HTS) method [13]. The amplicon sequence variant (ASV) method has many advantages when working with difficult samples that usually make specific sequence analyses. Operational taxonomic units (OTUs) are typically thought to be far more likely to retain unusual sequences when seeking to analyze low-abundance sequences results complete microbiome analysis [14]. Through their influence on the fundamental structure of the plant microbiome, seed microorganisms may significantly impact on crop productivity. However, seed-associated microorganism diversity and organization have frequently been disregarded, and seed endophytes are poorly characterized [15]. Additionally, seeds act as the first starter culture for the plant microbiome, passing bacteria from all the vegetative parts of the plant and from one generation to the next [15]. The seed microorganisms passed down through generations have a significant impact on the microecology, quality, and production of plants [16].

The main aim of the study is to identify the whole microbes present in both modern hybrids and traditional varieties and analyze their potential key players for further use in developing the consortia for plant health. In this study, the seeds and leaves of four traditional and two modern hybrids were used as research materials. High throughput sequencing was used to analyze the composition and diversity of endophytic microbiome (fungi and bacteria) in seeds and leaves of these traditional varieties and modern hybrids.

2. Materials and methods

2.1. Cucumber varieties

Six different varieties of cucumber were selected for the greenhouse pot studies. Among those two were hybrid (Seminis Malini, Hyveg Chitra) and four were traditional varieties (Agathiyar Heirloom, Spiny, Fruit, and Round cucumber) collected from farmers of different districts in Tamil Nadu, India.

2.2. Growth of plants

Totally 24 poly bags (24 × 24 cm) were taken for the study which were filled with a potting mixture of 250 kg farm soil (sandy loam texture), 125 kg vermicompost, 1.25 kg neem cake, 1.25 kg castor cake, 1.25 kg pungam cake, 0.375 kg diammonium phosphate and 0.250 kg Muriate of potash. All the seeds were sowed in this potting mixture with two seeds in each bag and allowed to grow in the greenhouse of Vellore Institute of Technology. The pH of the soil was 6.5, gritty texture and dark brown color. All the plants were maintained under 12 h uninterrupted sunlight and 12 h darkness with temperature maintained at 25–27 °C during the day and 18–20 °C during the night. The relative humidity was maintained with the help of sensors and sprinklers up to 60–70% during the day and 70–80% during the night.

2.3. Sample collection

In addition to the seeds that were collected from local farmers and then directly sampled for metagenome analysis, the leaf samples were collected when the plants were at the vegetative stage (40 days after the sowing of seeds collected from farmers). Metataxonomic analyses were carried out using 10 g of seed and leaf samples of each variety to identify bacterial and fungal endophytes.

2.4. Extraction of nucleic acid and metataxonomic analysis

Metataxonomic analysis was done by using paid facility at Biokart India Pvt. Ltd., Bangalore, India. The genomic DNA was extracted using Qiagen DNeasy Plant Mini Kit (Qiagen, Germany) as per the manufacturer's instructions and then the quality and quantity of nucleic acid was checked by using Nano drop Spectrophotometer (Thermo Scientific, USA) and agarose gel electrophoresis. Libraries were prepared using Illumina barcoded adapters, purified using Ampure beads, and quantitated using Qubit dsDNA High Sensitivity assay kit.

Sequencing was performed using Illumina Miseq with 2x300PE v3 sequencing kit. The obtained raw sequences were deposited in the SRA database in NCBI with the following accession numbers 1. Seed 16s (SAMN23380189, SAMN2338010190,

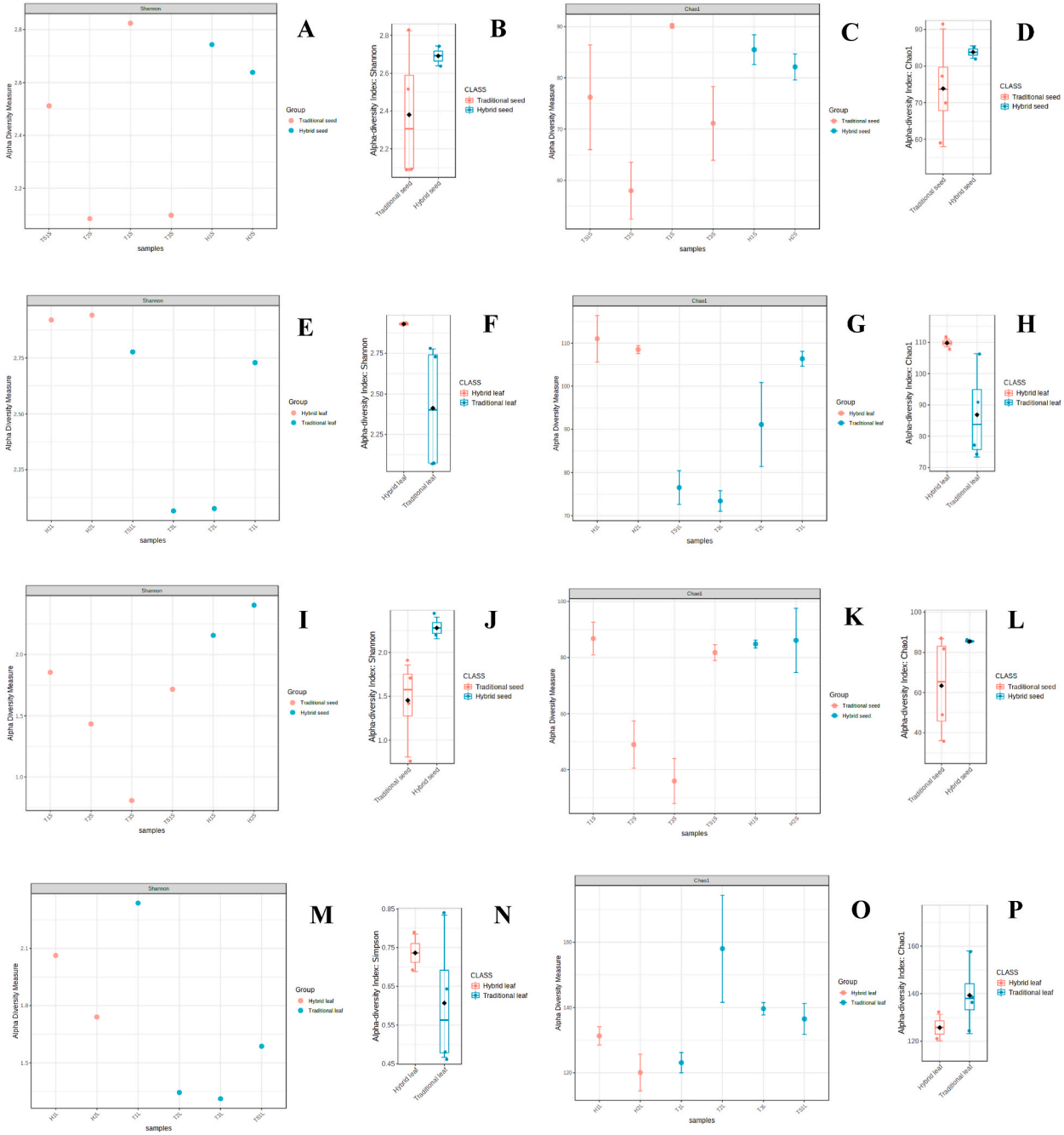

Fig. 1. α-diversity of the endophytic bacteria and fungi in leaves and seeds of traditional varieties and hybrids of cucumber
Based on the Shannon and Chao1 index, samples demonstrate variable evenness and richness of bacterial diversity on the X and Y, respectively. The Kruskal-Wallace test, which has $P < 0.05$, is also used to determine the statistical significance of the grouping based on source (Seeds of traditional varieties = Peach color; Seeds of hybrids = Blue color and Leaves of traditional varieties = Blue color; Leaves of hybrids = Peach color).
Fig. 1A and B represent the endophytic bacterial diversity in seeds using Shannon indexing and box plots; Fig. 1C and D represent the Chao1 and box plots. Fig. 1E and F represent the endophytic bacterial diversity in leaves of varieties using Shannon indexing and box plot. Fig. 1G and H represent the Chao1 and box plots. Fig. 1I and J represent the endophytic fungal diversity in seeds of varieties using Shannon indexing and box plots. Fig. 1K and L represent the Chao1 and box plots. Fig. 1M and N represent the endophytic fungal diversity in leaf tissues of varieties using Shannon indexing and box plots. whereas Fig. 1O and P represent the Chao1 and box plots.

SAMN2338010191, SAMN2338010192, SAMN2338010193, SAMN2338010194); 2. Seed ITS (SAMN23401314, SAMN23401315, SAMN23401316, SAMN23401317, SAMN23401318, SAMN23401319); 3. Leaf 16s (SAMN23394854, SAMN23394855, SAMN23394856, SAMN23394857, SAMN23394858, SAMN23394859); 4. Leaf ITS (SAMN23394000, SAMN23394001, SAMN23394002, SAMN23394003, SAMN23394004, SAMN23394005).

2.5. Bioinformatic analysis

The raw data quality check was done using FASTQC and MULTIQC, followed by trimming of adapters and low-quality reads with a default threshold value below 20 by TRIMGALORE. The trimmed reads were further taken for processing, including merging of pair-ends, chimera removal, and OTUs abundance calculation and the estimation correction achieved by QIIME workflow. This workflow enabled highly accurate investigations at the genus level. The database used was NCBI.

The alpha diversity was measured by using Shannon indexing (Provides information about both richness and evenness of species in a sample) and Chao1 (estimates the total species richness) estimate in a alpha diversity tool. Chao1 estimate the richness by inferring out the number of rare organisms that may have been lost due to under sampling. Shannon index is used to describe the actual diversity of a community. Depending on Chao1 and Shannon with a significance level $< P\ 0.05$ findings, the bars in the whisker box show the range of least and highest diversity values within a population, omitting outliers that exist as single points.

Ordination-based method Principle Coordinate Analysis (PCoA) was used to visualize the beta diversity of samples in 2D plot where each point represents the entire microbiome of a single sample. Each axis reflects the percent of the variation between the samples with the X-axis representing the highest dimension of variation and the Y-axis representing the second highest dimension of variation. Also, the statistical significance of the clustering pattern in ordination plots can be evaluated using Permutational ANOVA (PERMANOVA). The heatmaps were generated using heat map function in R and QIIME to reveal the relative abundance, prevalence and metabolism of microbial diversity. A data matrix is displayed using a heatmap, and the coloring provides a summary of numerical differences.

SparCC algorithm was used to build this correlation network, and box plots which makes an assumption of a sparse network (i.e

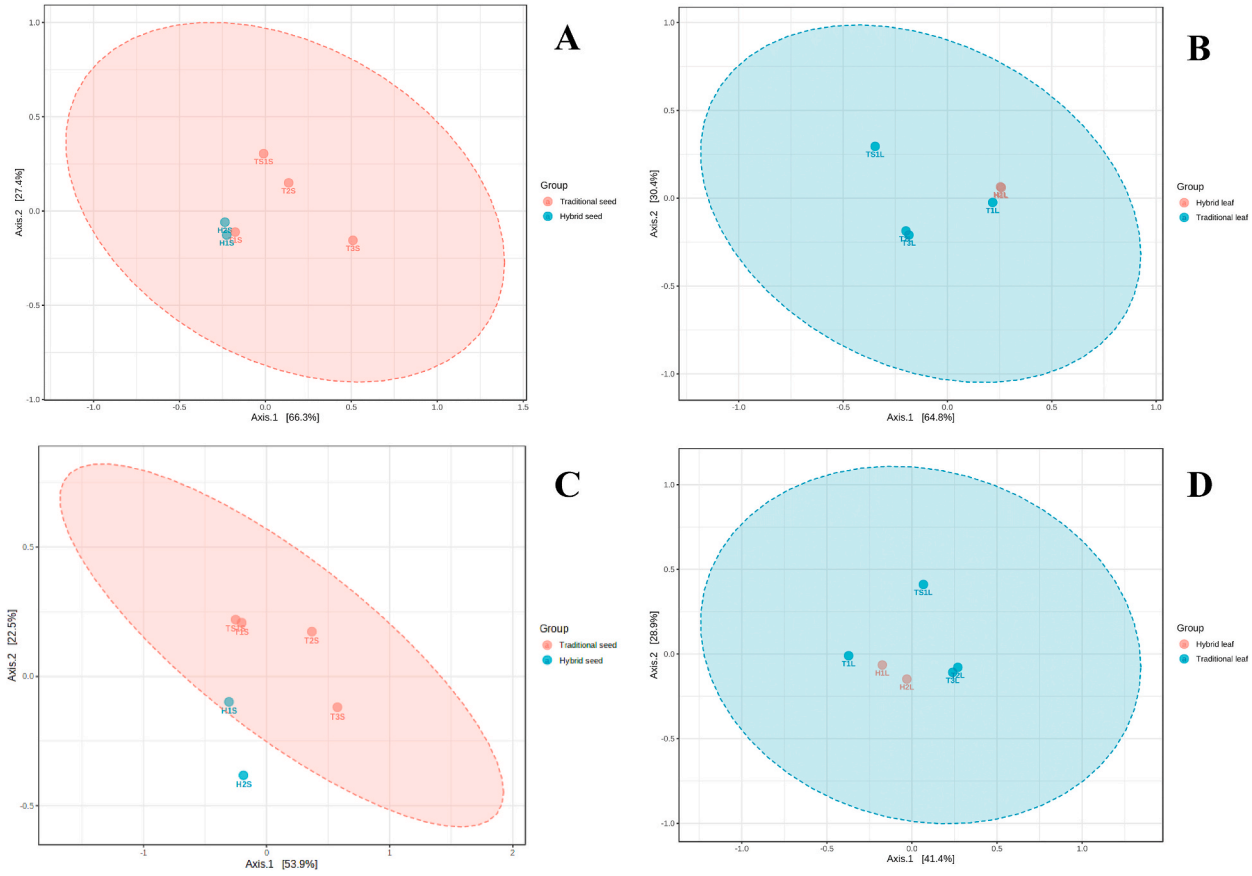

Fig. 2. PCoA of the endophytic bacteria and fungi in leaves and seeds of traditional varieties and hybrids of cucumber
Fig. 2A: Endophytic bacteria in seeds. Fig. 2B: Endophytic bacteria in leaves. Fig. 2C: Endophytic fungi in seeds. Fig. 2D: Endophytic fungi in leaves. Samples with different diversity were not clustered. Results are presented as 2D ordination plots based on principle coordinate analysis (PCoA). The corresponding statistical significance is assessed using permutational multivariate analysis of variance (PERMANOVA). Samples displayed on the PCoA plots are colored according to the image legends.

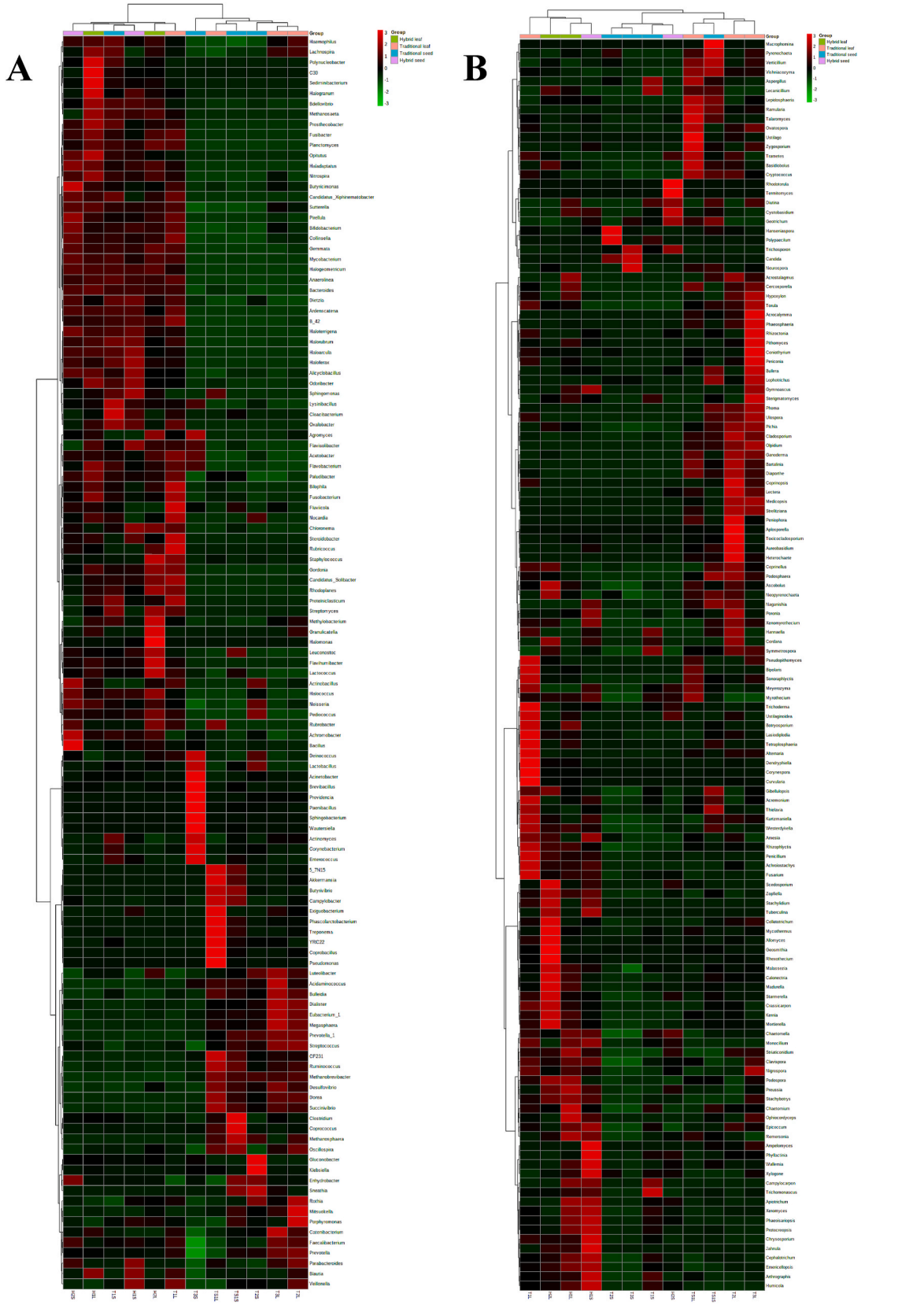

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Fig. 3. Heat maps of bacterial and fungal genera in leaves and seeds of traditional varieties and hybrids of cucumber
Fig. 3A. Heat map of fungal genera. Fig. 3B. Heat map of bacterial genera. Depending on row z-scores, the heat map is colored. Bright red has a strong correlation (r = 3), while bright green has a strong adverse correlation (r = −3). The samples are categorized in the legends by different colors according to how frequently certain genera occur.

that many taxa are not correlated with one another) and uses a log ration transformation and performs iterations to identify taxa pairs that are outliers to background correlations. Circos plot was generated in Circos tool by using relative abundance of core microbiome.

3. Results

3.1. Microbial diversity in seeds and leaves of cucumber varieties and hybrids

3.1.1. Alpha diversity

Using this technique, the level of diversity existing in a sample or group can be assessed. The overall richness of species, the evenness of the species, or measurements that took both richness and evenness into account can be used to describe alpha diversity. The work was carried out to evaluate and compare the abundance of endophytic bacterial and fungal communities between the hybrid and traditional varieties of cucumber (Fig. 1). These statistics, which are derived from Kruskal-Wallace, Chao1, and Shannon $P < 0.05$ results combined, show that the microbial diversity in modern hybrid varieties varies highly in microbial diversity with traditional varieties in leaf as well as seed tissues. The bars in the whisker box depict the range of least and highest α-diversity values within a population excluding the outliers that occur as single points.

Seed endophytic bacterial diversity: Shannon and Chao1 indexes revealed that the endophytic bacterial diversity is higher in seeds of traditional variety T1S and hybrid H1S whereas the lowest diversity was seen in seeds of traditional varieties T2S and T3S (Fig. 1A and B). The bacterial richness was greatest in seeds of traditional variety T1S and hybrid H1S whereas lowest in seeds of traditional variety T2S (Fig. 1C and D).

Leaf endophytic bacterial diversity: The endophytic bacterial diversity was found to be higher in leaves of hybrid cucumbers (H1L and H2L), whereas the traditional varieties (T3L and T2L) showed the lowest diversity (Fig. 1E and F). The hybrid H1L and traditional variety T1L showed the greatest richness, whereas the lowest was seen in the traditional variety T3L (Fig. 1G and H).

Seed endophytic fungal diversity: The hybrids H2S and H1S showed the highest diversity, whereas the lowest diversity and richness were observed in the seeds of the traditional variety T3S (Fig. 1I and J). However, the seeds of hybrid H2S and the traditional variety T1S showed the highest richness (Fig. 1K and L).

Leaf endophytic fungal diversity: In the case of fungal diversity in leaf tissues, the highest and lowest endophytic fungal diversity was observed in traditional varieties. The highest diversity in variety T1L and the lowest in variety T3L (Fig. 1M and N). The overall richness is more in traditional variety T2L and the lowest in the hybrid H2L (Fig. 1O and P).

3.1.2. Beta diversity

The findings of beta diversity of hybrid and traditional varieties indicate that every sample is distinct and that the abundance and diversity of OTUs vary with respect to one another. These matrices were visualized in a 2D plot using the ordination-based approach Principle Coordinate Analysis (PCoA), where every point represented the whole microbiota of a single sample (Fig. 2).

Beta diversity of the endophytic bacterial communities in seeds: In connection with the endophytic bacterial communities of seeds, Fig. 2A represent a group consisting of the hybrids H1S, H2S and the traditional variety T1S discussing their close relationship and similar diversity. Seeds of traditional varieties TS1S, T2S and T3S showed unique diversities.

Beta diversity of the endophytic bacterial communities in leaves: Fig. 2B represents the leaves of H1L and H2L hybrids grouped together, which means they showed similarity in species diversity. An identical pattern was seen in leaves of traditional varieties T2L and T3L, whereas the traditional varieties T1L and TS1L showed unique diversity and similarity among them.

Beta diversity of the endophytic fungal communities in seeds: In connection with the endophytic fungal communities of seed verities, Fig. 2C represents the TS1S and T2S traditional varieties grouped together, demonstrating their close relationship and corresponding species diversity. There was no identical pattern observed in other varieties. The remaining traditional seed verities T2S, T3S, and hybrids H1S and H2S showed unique diversity and similarity among them.

Beta diversity of the endophytic fungal communities in leaves: Fig. 2D represents the diversity in leaves of T3L and T2L varieties grouped together, which means they showed indistinguishable species diversity. Traditional variety T1L and hybrids H1L and H2L failed to form a group but exhibited closer diversity with each other. Traditional variety TS1L showed unique diversity and dissimilarity of species compared to the rest.

3.2. Heatmap of bacterial and fungal genera

The heap map shows the relative abundance of bacterial and fungal species based on the positive and negative correlations. The heatmap revealed the greater abundance of different bacterial genera which was found in all the groups (Fig. 3). The bacterial genus *Bacteroides* was abundant in all groups whereas, the genus *Lactobacillus* was mainly predominant in the seeds of traditional varieties of cucumber. *Prevotella*, *Dialester*, and *Fecalibacterium* were predominant in the group of traditional leaf verities (Fig. 3A). Among the fungal genera, *Pichia* was abundant in the leaves of traditional varieties, whereas *Candida* showed a higher abundance in the traditional

seed group. *Aspergillus* and *Malassezia* were found to be abundant in the hybrid leaf group whereas *Phaeoisariopsis* showed a higher abundance in the hybrid leaf as well as seed group (Fig. 3B).

3.3. Core microbiome of cucumber leaves and seeds

Core microbiome represents the genomes which are common to all the samples studied. Cluster microbiomes were generated for bacteria and fungi where genes are represented in leaves and seeds (Fig. 4). *Prevotella*, *Bacteroides*, and *Faecalibacterium* showed the highest prevalence with 1.0% whereas *Dialister* and *Lactobacillus* showed the prevalence of 0.9% and 0.5% respectively (Fig. 4A). The fungal genera *Pichia* showed the highest prevalence with 1.0%. *Malassezia* and *Aspergillus* showed a prevalence of 0.9% and 0.8% respectively, followed by 0.5% for *Phaeoisariopsis* and 0.4% for *Candida* (Fig. 4B).

3.4. Heatmap of bacterial metabolic functions

Metabolic functions in seeds: Bacterial metabolic functions were predicted based on the number of OTUs capable of each function, by targeting the gene which is meant for a specific function. Bacterial genera in seed of traditional varieties showed higher metabolic functions than the bacteria in hybrids (Fig. 5). The variety T3S had abundant gramicidin producer, sulphur metabolizing, chlorophenol degrading, aromatic hydrocarbon degrading and sulphur oxidizing bacteria. The variety T2S had dehalogenation and ammonia oxidizers, whereas T3S had xylan-degrader and T1S had oxalic acid degrader and selenate reducer. However, the hybrid variety H2S showed abundant streptomycin producers which were not found in traditional varieties.

Metabolic functions in leaves: Bacterial genera in leaf tissues of varieties showed a similar trend as the seeds (Fig. 5). Leaf of the traditional variety TS1L had abundant cellulose degrader, methanogen, chitin degradation, nitrogen fixation, and atrazine metabolism, whereas the leaf of hybrid H2L showed the napthelene degrading metabolism which was not found in leaves of traditional varieties.

3.5. Co-network analysis of bacteria and fungi identified in the seed and leaf metagenome of cucumber

Co-network analysis was carried out to understand the interactions within bacterial species and within fungal species. The species which have more number of networking partner species might play key role in the effects of microbiome on the plant's performance. The identification of a high number of taxa and the prediction of key species and their interactions was aided by microbial network analysis (Fig. 6). The correlation of taxonomic features is presented in Fig. 7. *Bacteroids, Lactobacillus, Oxalobacter, Paenibacillus*, and *Succinivibrio* were found to be a high number of taxa and may be key players of seed microbiome in both traditional varieties and hybrids (Fig. 6A). All the taxa features in both traditional varieties and hybrids were correlated except *Oxalobacter* in the seeds of traditional variety (Fig. 7A). In leaves, *Prevotella, Bacteroides, Lactobacillus, Dialester*, and *Halorcula* were found to be a high number taxa and may be key players of leaf microbiomes in traditional varieties and hybrids (Fig. 6B). All the taxa features in both traditional varieties and hybrids were correlated except *Lactobacillus* and *Halorcula* in leaves of traditional varieties (Fig. 7B).

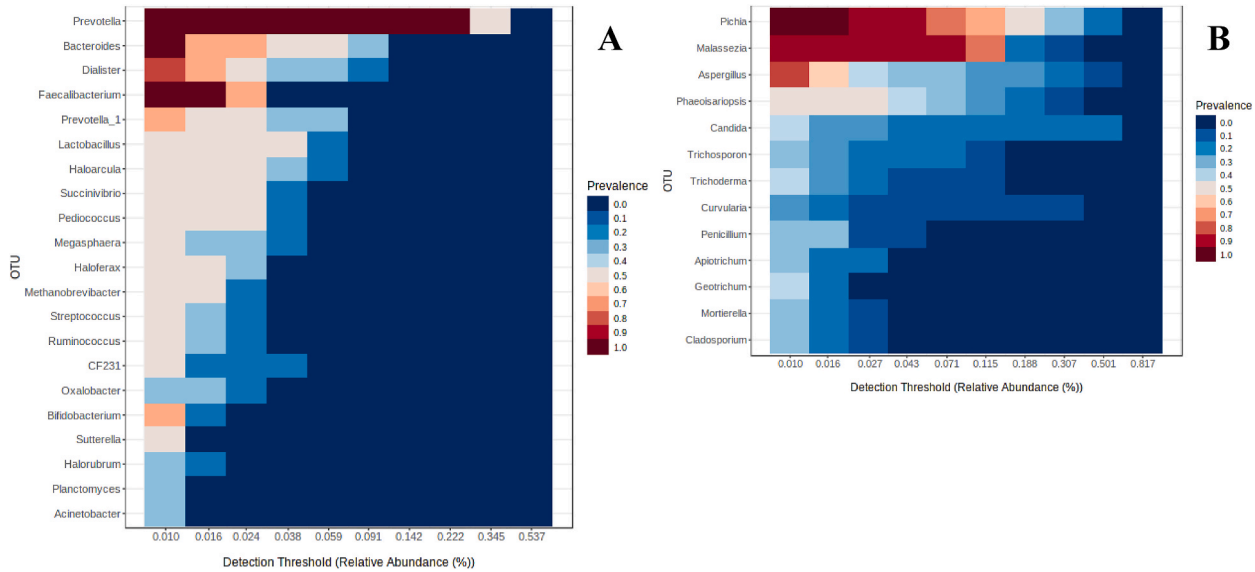

Fig. 4. Heat map of the core microbiome of cucumber (fungi and bacteria).
Detection threshold used was 10% for predominance and 0.1% for relative abundance. Fig. 4A: core microbiome – bacteria. Fig. 4B: core microbiome – fungi.

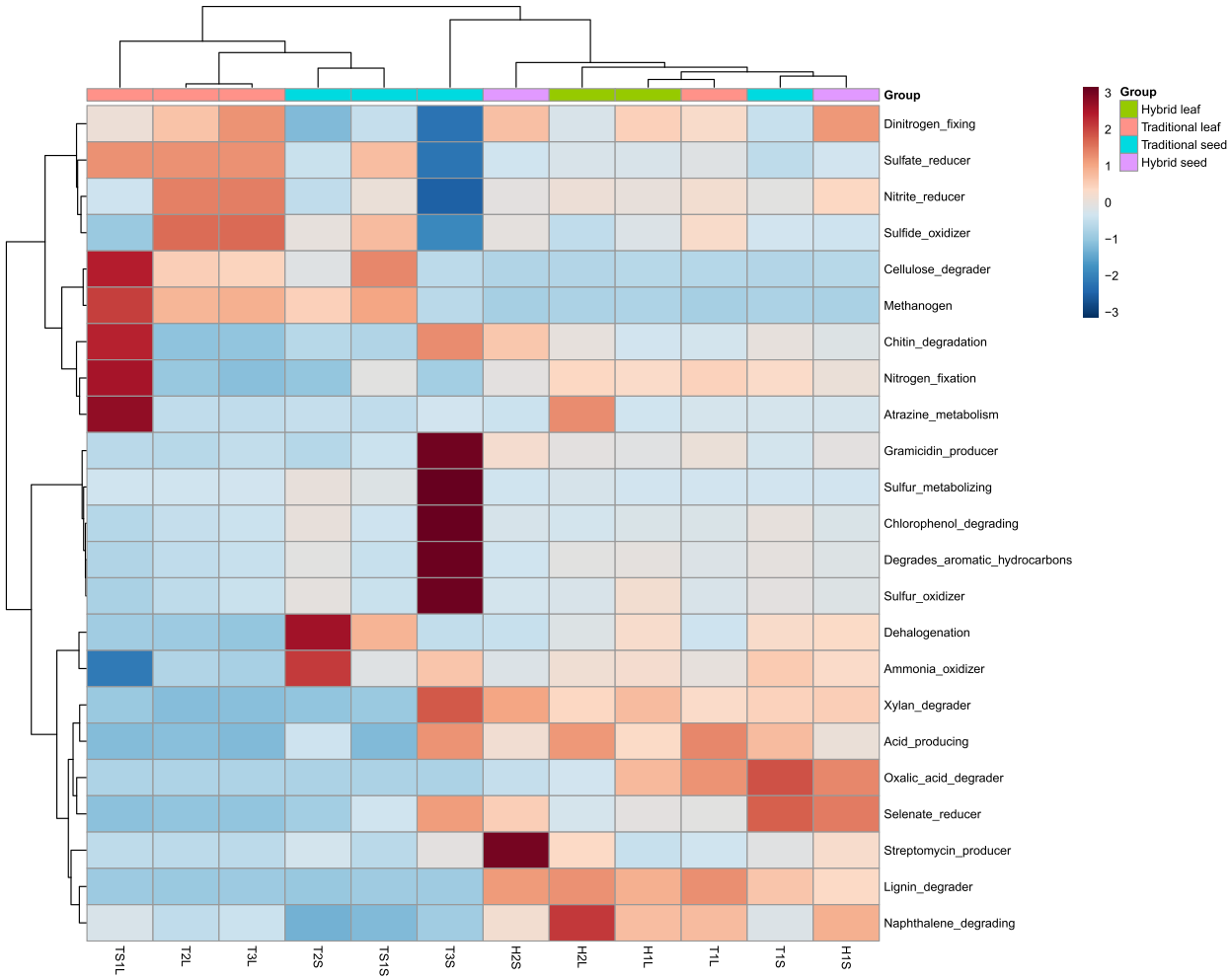

Fig. 5. Heatmap of bacterial metabolism in seeds and leaves of traditional varieties and hybrids of cucumber The heat map depicts the number of OTUs responsible for a specific gene function from both seed and leaf samples. Bright red has a strong correlation (r = 3), while bright blue has a strong adverse correlation (r = −3).

With regard to fungi, *Pichia, Aspergillus, Candida, Phaeoisariopsis*, and *Polypaecilium* were found to be a high number taxa and may be key players in seed microbiomes of traditional varieties and hybrids (Fig. 6C). All the taxa features were correlated except *Pichia* and *Aspergillus* in seeds of traditional varieties (Fig. 7C). In leaves, *Pichia, Aspergillus, Phaeoisariopsis, Curvularia*, and *Cladosporium* were found to be a high number taxon (Fig. 6D). All the taxa features in traditional varieties and hybrids were highly correlated (Fig. 7D).

3.6. Circos plot analysis of bacterial and fungal diversity based on OTUs

In order to visualize the relative abundance of different bacterial and fungal communities in the cucumber microbiome, Circos plot analysis was carried out using the OTU count of the individual genera in the metataxonomic data. Results of the analysis are given in Tables 1 and 2. In terms of relative abundance, bacterial genera in seeds of traditional varieties showed the highest abundance with OTUs of *Lactobacillus* (12,069), *Prevotella* (4,297), *Dialester* (406), and *Faecalibacterum* (245) in comparison with seeds of hybrids which showed *Bacteroides* (639). Bacterial genera in leaves of traditional varieties followed the highest abundance like seed varieties with OTUs of *Prevotella* (18,538), *Dialester* (3,975), *Bacteroides* (1,570), and *Faecalibacterium* (1,333). *Lactobacillus* (710) dominated in leaves of hybrid cucumbers (Fig. 8A).

Fungal genera in seeds of traditional varieties showed the highest abundance with OTUs of *Candida* (1,82,175), *Aspergillus* (57,296), *Pichia* (19,858), and *Malassezia* (16,698). *Phaeoisariopsis* (24,913) dominated in seeds of hybrids. Fungal genera in leaves of hybrids showed the highest abundance than traditional verities with OTUs of *Malassezia* (45,382), *Phaeoisariopsis* (39,529), and *Candida* (381). *Pichia* (38,300) and *Aspergillus* (22,746) were found to be abundant in the leaves of traditional varieties (Fig. 8B).

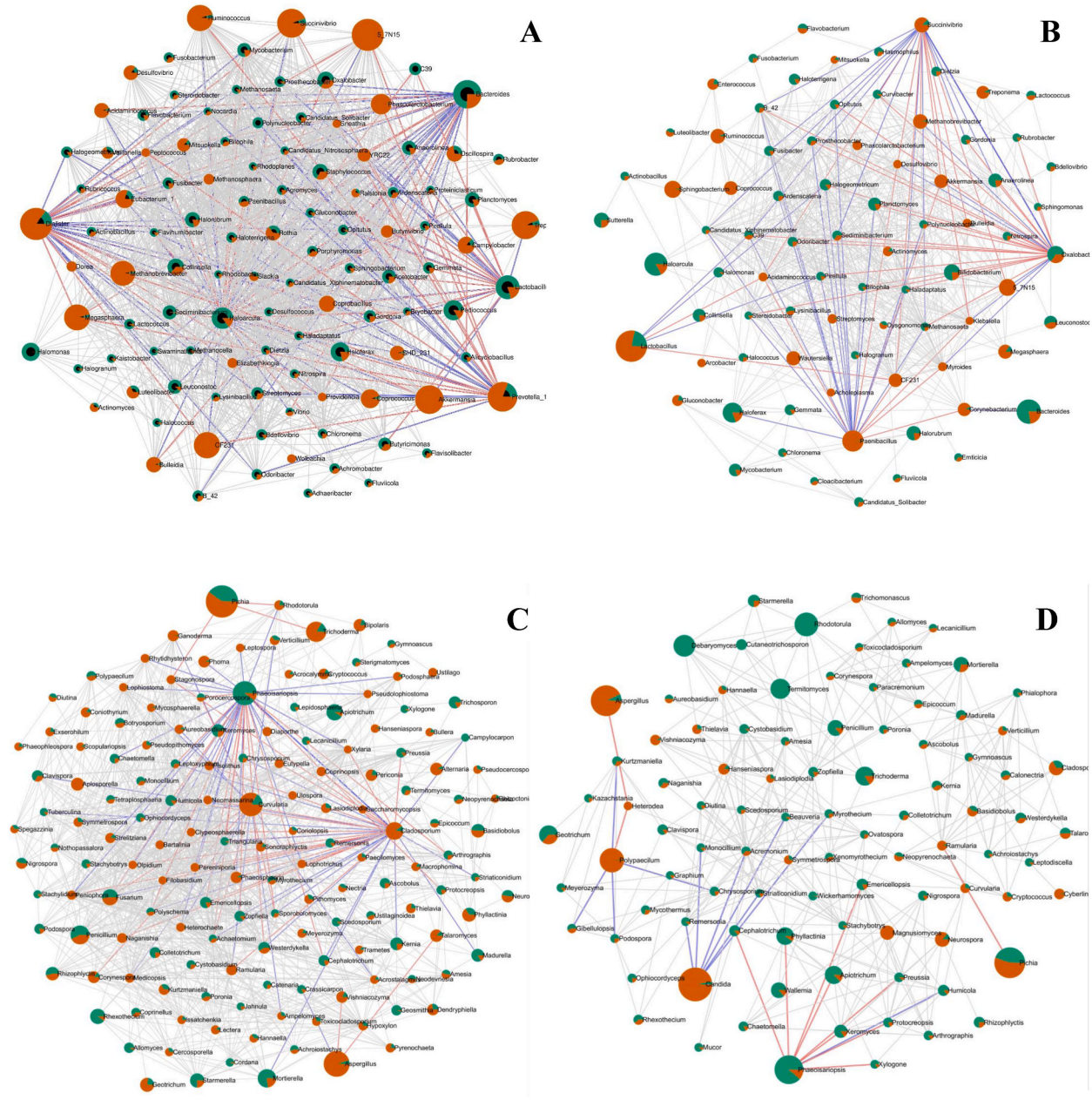

Fig. 6. Network analysis in bacteria and fungi in seed and leaf microbiome of traditional varieties and hybrids of cucumber A. Bacteria in seeds B. Fungi in seeds C. Bacteria in leaves D. Fungi in leaves.

4. Discussion

Recently plant endophytes received more attention to improving the quality of agricultural crops. Microbiome of cucumber plants are yet to be studied in depth. Chen et al. [17] studied the declining structure of rhizosphere microbiome of cucumber and the soil metabolic function due to continuous cultivation in same soil under greenhouse conditions. Zhou et al. [18] reported the changes in diversity of rhizosphere microbiome in cucumber due to variations in artificial substrates used for cultivation. The composition of endophytic microbial communities in cucumber plants was also reported to change with growth stages [19]. Profiling the endophytic microbiome of different plant parts like seeds, leaves etc is essential to understand the varying composition and functions of endophytic microbes. In addition, comparison of endophytic microbiome of traditional varieties and modern hybrids will help to explore the beneficial phenotype specific microbes for future microbiome engineering. With the help of metataxonomic sequencing, the current study revealed many cultured and uncultured bacterial and fungal plant endophytes in the seed and leaf tissues of traditional varieties and modern hybrids of cucumber.

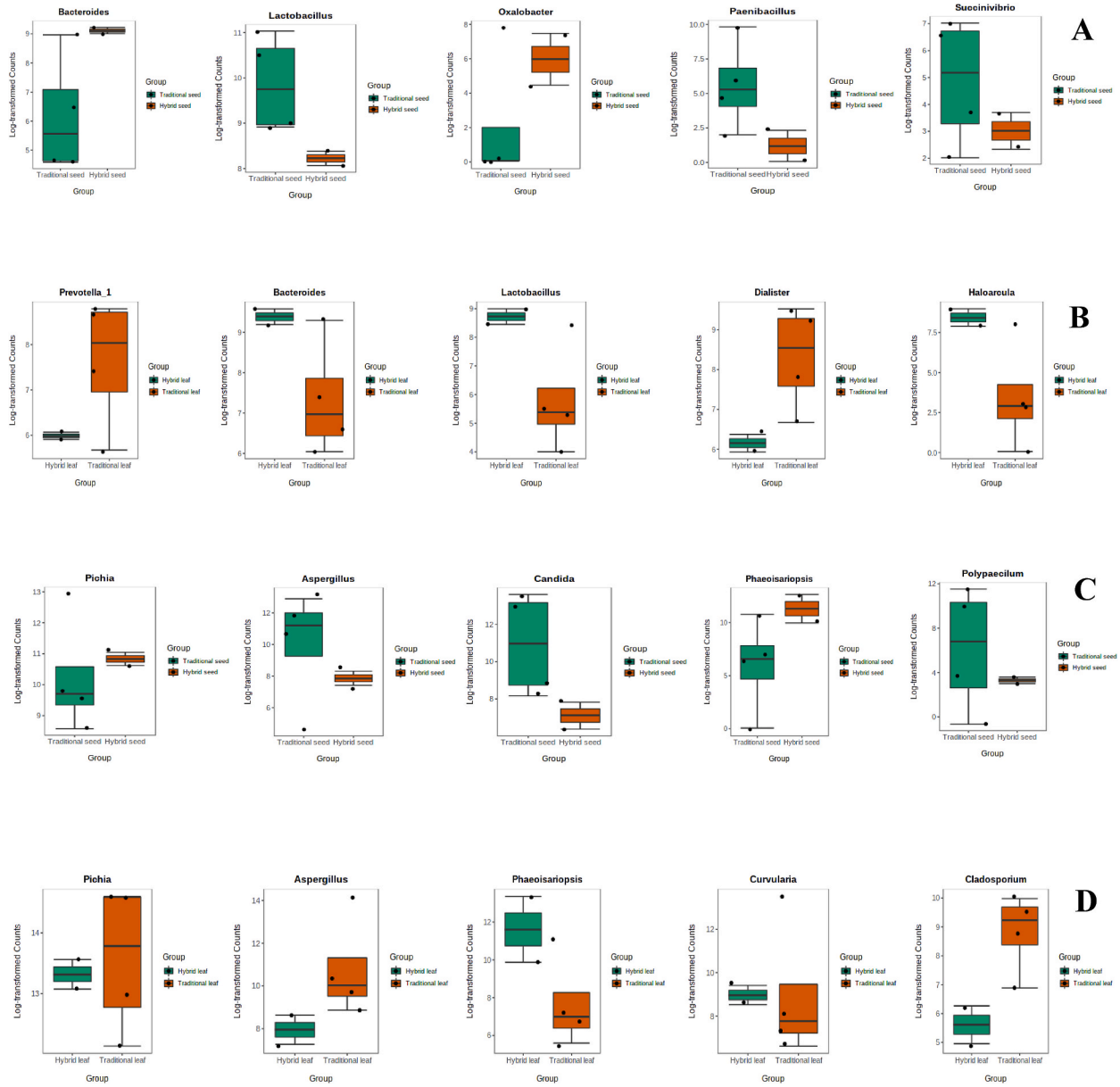

Fig. 7. Box plots indicating the top 10 features of correlation in taxa in cucumber seed and leaf microbiome A. Bacteria in seeds B. Fungi in seeds C. Bacteria in leaves D. Fungi in leaves.

The diversity and richness of bacteria were found to be more in leaves and seeds of hybrid cucumber, whereas the fungal diversity and richness were found to be highest in leaves of traditional varieties. In hybrid seeds, both fungal and bacterial diversity is more compared to traditional varieties. The similarity of bacterial and fungal diversity varied in all the traditional varieties. In hybrids, the diversity of bacteria, and in traditional varieties the diversity of fungi was similar in both seeds vs. leaves.

Based on the core microbiome, certain endophytic bacterial and fungal genera were found to be uniquely present either in traditional varieties or the hybrids, but not in both. The fungal genera like *Trichoderma, Curvularia,* and *Cladosporium* are present only in traditional varieties whereas *Apotrichum* and *Mortierella* are present only in hybrids. Although the richness and diversity of bacteria were more in hybrids in the overall microbiome, Considering the species in core microbiome, traditional varieties have a greater number of bacteria. While *Haloarcula* was present only in hybrids, bacterial genera in traditional varieties include *Prevotella, Succinovibrio, Megasphaera, Methanobrevibacter, Streptococcus, Ruminococcus,* and *Acinetobacter.* The major genera reported by Ref. [19] in the cucumber rhizosphere were *Bacillus* and *Sphingomonas* (bacteria), *Chaetomium*, *Mortierella*, *Aspergillus* and *Penicillium* (fungi).

Some of the endophytic microbes reported in cucumber are: *Chryseobacterium* [20], *Chaetomium* [21], *Paenibacillus* [22], *Paecilomyces* [23], *Saccharothrix* [24], *Aspergillus, Penicillium, Fusarium, Beauveria, Trichoderma, Colletotrichum, Cladosporium, Alternatia,*

Table 1
Operational taxonomic units (OTUs) of major bacterial genera in seed and leaf microbiome of cucumber traditional varieties and hybrids.

Genus	H1L	H2L	T1L	T2L	T3L	TS1L	H1S	H2S	T1S	T2S	T3S	TS1S
Prevotella	3185	3157	2729	9915	18538	14193	2179	1999	2440	4297	3858	3544
Bacteroides	102921	840	587	258	384	1570	639	581	703	63	80	158
Dialester	92	118	86	1774	3975	2177	57	75	69	406	246	302
Faecalibacterium	243	222	199	649	1333	859	172	183	169	234	205	245
Prevotella_1	90	95	60	1243	2445	1672	63	48	60	495	412	481
Lactobacillus	510	747	344	127	211	201	396	292	658	3075	12069	806
Haloarcula	710	332	254	14	43	25	752	369	557	7	10	4
Succinivibrio	20	14	12	668	1277	2970	16	6	7	185	133	269
Pediococcus	308	476	207	6	14	7	187	187	200	629	32	10
Megasphaera	8	7	7	669	1528	853	12	6	16	178	109	118
Haloferax	313	215	177	12	32	78	400	185	337	9	5	8
Methanobrevibacter	7	1	1	353	694	1482	2	2	5	166	129	160
Streptococcus	69	61	58	531	878	614	51	47	42	200	77	176
Ruminococcus	12	5	5	361	637	2163	8	0	4	98	84	246
CF231	0	0	0	246	478	2536	0	0	0	96	92	215
Oxalobacter	215	61	206	4	13	5	220	25	299	0	1	0
Bifidobacterium	156	134	131	127	318	171	97	113	112	27	29	29
Sutterella	134	108	96	124	276	158	75	89	86	34	28	31
Halorubrum	132	84	78	3	17	8	109	62	118	0	4	2
Planctomyces	119	98	68	5	11	5	58	55	67	1	4	2
Acinetobacter	96	76	68	91	227	61	76	52	98	169	2871	52

H – Hybrid; T – Traditional variety; L – Leaf; S - Seed.

Table 2
Operational taxonomic units (OTUs) of major fungal genera in seed and leaf microbiome of cucumber traditional varieties and hybrids.

Genus	H1L	H2L	T1L	T2L	T3L	TS1L	H1S	H2S	T1S	T2S	T3S	TS1S
Pichia	32881	35887	4729	24373	38300	9812	8458	1639	6736	3183	5646	19858
Malassezia	32694	45382	5557	4533	7967	4675	11221	2043	16698	6071	888	7351
Aspergillus	1439	482	826	1272	746	22746	1129	190	57296	6651	401	9263
Phaeoisariopsis	39529	2852	2407	50	135	200	24913	1028	12894	377	25	176
Candida	381	205	125	79	248	114	897	76	3685	34535	182175	759
Trichosporon	725	12	21	17	42	20	586	1516	2760	939	26737	160
Trichoderma	1521	139	4198	71	451	388	1183	574	1852	45	58	263
Curvularia	2662	1154	11275	280	260	107	9	1	5	1	7	51
Penicillium	1947	2665	1871	92	193	108	1018	90	344	14	21	209
Apiotrichum	5053	61	91	63	93	44	2310	188	1252	41	10	121
Geotrichum	330	8	65	47	86	554	591	381	748	16	2499	916
Mortierella	2037	4275	533	217	381	258	626	82	314	43	102	370
Cladosporium	294	109	111	1006	1183	560	174	25	347	41	84	607

H – Hybrid; T – Traditional variety; L – Leaf; S - Seed.

Chaetomium [25], *Fusarium, Chaetomium, Colletotrichum, Acrocalymma* [26]. The *Pencillium, Gignardia, Hypocrea, Neurospora, Eupenicillium, Lasiodiplodia* observed by Abro et al. [27] were found to have antagonistic activity against *Fusarium oxysporum* f.sp. *Cucumerinum* causing wilt in cucumber. These fungi and bacteria are effective and many of them possess plant growth-promoting activities, but due to continuous cropping or development of hybrids with disease resistance might have led to removal of the traditional microbes.

Bioinformatic network analysis was performed to understand the networking of microbial communities observed in the datasets. Among bacteria, *Bacteroides* and *Lactobacillus* showed more networking with other bacteria in both hybrid and traditional varieties (seeds and leaves). *Prevotella* alone showed the highest networking in leaves of traditional varieties as well as hybrids. Among fungi, *Pichia*, *Aspergillus* and *Phaeoisariopsis* showed more networks in both seeds and leaves of traditional varieties and hybrids, whereas *Candida* mainly showed networking in seeds of traditional varieties. These networks may or may not exist in the cucumber seeds or leaves as the analysis is based on data from previous reports. However, the results could provide some guidance for exploring these microbes for future work.

The metabolism heat map indicates the seeds of hybrid (H2S) have microbes that can produce antibiotic streptomycin. These organisms might have been recruited as an endophyte due to continuous cultivation disease pressure. Leaves of the same hybrid (H2L) was found to have microbes that could degrade naphthalene, attributing to the plant's possible ability to survive in contaminated environment. Seeds of traditional varieties (T3S, T2S, T1S) have gramicidin producers, sulphur and ammonia oxidizers, xylan and oxalic acid degrader etc. Leaves of traditional variety (TS1L) has bacteria involved in nitrogen fixation and chitin degradation. This could be because the traditional varieties are not majorly cultivated and are more tolerant towards stress metabolism naturally.

Among the bacterial genera observed in the present study, the phylum *Bacteroidetes* and *Bacillota* dominated the whole cucumber plant microbiome in all cultivars. *Prevotella* and the *Bacteroides* genus belongs to *Bacteroidetes* which are clinically significant

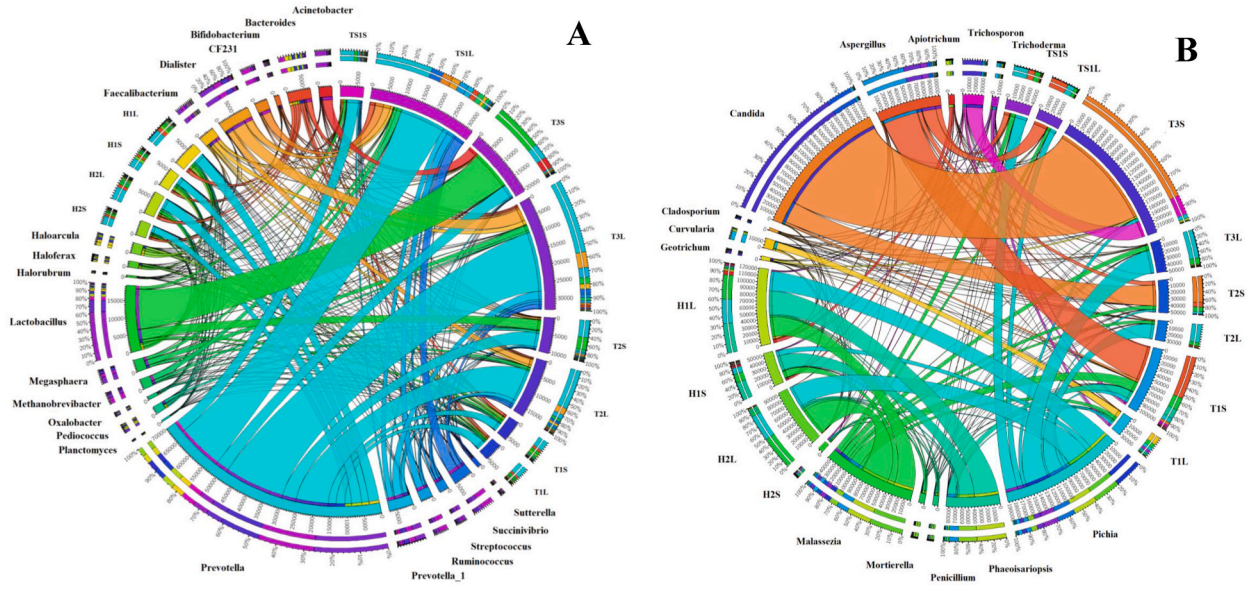

Fig. 8. Circos plot analysis of bacterial and fungal diversity in cucumber microbiome based on OTUs
The arcs with a particular colour show the relationship between the relative abundance of the microbial community and the samples. The ribbon-shaped area denotes each variable's contribution and shows a strong positive association. A. Circos plot of bacteria B. Circos plot of fungi.

enterotypes and are primarily found in humans' mouth, stomach, and vagina. Individuals who eat a fiber-rich vegetable diet have high amounts of *Prevotella,* whereas people who eat meat have high loads of Bacteroides. These microorganisms are effective at breaking the hard fibers present in plant diets. According to Arumugam et al. [28], there are various phylogenetic and functional variances amongst enterotypes, *Prevotella* is recognized as a mucin degrader, but *Bacteroides* spp have a broad saccharolytic capability (degradation of galactosidases, proteases, and hexosaminidases). Complex arabinoxylans from dietary fiber are broken down by *Bacteroides* spp., which releases ferulic acid [29]. Ferulic acid has been shown to have positive health impacts thanks to its antioxidant properties, immune system, inflammatory response, and cell survival-modulating abilities [30]. *Lactobacillus*, *Dialester*, and *Fecalibacterium* belong to the phylum *Bacillota* which are major genera in the human gut and soil. All these bacteria come under vertebrate gastrointestinal microbiota and are important in maintaining human gut health [31–33].

Mu et al. [34] stated that *Lactobacillus* species can benefit the immune system and able to restrict the spread of harmful bacteria and changing the composition of the commensal microbiome. Numerous illnesses, such as vaginosis caused by bacteria, upper respiratory tract infections, and dermatitis, have been successfully treated with lactobacilli [35]. *Faecalibacterium* species consume acetate and generate butyrate as well as functional anti-inflammatory compounds including shikimic and salicylic acids, which are necessary for the synthesis of vitamins [36]. These species are acknowledged as a vital component of the gut which significantly affects the host's metabolism and overall health. The current research showed the presence of a large number of *Bacteroidetes* and *Bacillota* phylum in cucumber plants which means consuming traditional varieties or hybrid of cucumbers helps to attain good gut health and increase immunity. However, the drawback behind having these bacteria in gut might lead to a few common infections which are not greatly notable in comparison with health benefits.

However, these bacteria have been reported to have different roles in plant hosts, Malviya et al. [37] described that 16S rRNA data revealed that *Prevotella* is one of the major genera in different sugarcane species. A review of the literature indicated that *Prevotella* has the *nif*H gene for nitrogen fixation. According to this, the presence of several *Prevotella* species promotes plant growth and can be employed as a nitrogen fixer during the cultivation of other crops. *Bacteroides* promote the mobilization of phosphorus and function as a natural inhibitor to numerous pathogens. Lidbury et al. [38] found that all *Bacteroidetes* associated with plants display persistent phosphatase activity.

Lactobacilli can encourage plant growth by generating volatile amino acids, auxins, and plantericin. They are also being used as biocontrol agents for having antifungal action and to combat plant-pathogenic bacteria in soil [39]. In the cucumber plants, inoculation of *Lactobacilli* bioformulation was reported to increase 17 different amino acids thereby promoting plant growth [40]. It is a prominent bacterium for plant growth promotion and disease control for many decades in agricultural practices. *Dialester* and *Faecalibacterium* are not explored in plant research. The presence of these bacteria could indicate that they play a role in plant growth.

Among fungal genera observed in the present study, *Pichia* is mainly present in healthy humans and fights against *Candida* which causes deadly infections in the body [41]. *Malassezia* is a superficial dimorphic fungus mainly found in human skin and causes skin diseases [42]. Since it is observed for the first time in cucumber in the current study, its possible plant growth-promoting activity is yet to be studied.

The fungal genus *Pichia* showed domination in all the varieties and hybrids. This genus is known to enhance plant hormone Indole acetic acid in wheat staw-based production medium [43] moreover *Pichia* improved the soil's physical, chemical, and biological

properties [44]. *Candida* is a potential biocontrol agent and a plant growth promotor [45]. For decades, *Aspergillus* is known for its plant growth-promoting ability and systemic resistance against crop diseases. *Phaeoisariopsis* is mainly a phytopathogen that causes angular leaf spots [46,47] as well as a plant growth promoting rhizosphere bacteria [48].

5. Conclusions

Based on the importance of the bacterial and fungal genera in the growth and yield of cucumber plants, the following genera are proposed as key members of the cucumber microbiome. This includes five bacterial genera *Prevotella, Bacteroides, Lactobacillus, Dialester, and Fecalibacterium,* and five fungal genera *Pichia, Aspergillus, Phaeoisariopsis, Candida,* and *Malassezia.* Further exploration of their individual and relative roles in cucumber growth, disease resistance, and yield would help to develop a microbial consortium for microbiome engineering in cucumber.

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Author contribution statement

Botlagunta Navya: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Subramanian Babu: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data associated with this study has been deposited at SRA database in NCBI with the following accession numbers 1. Seed 16s (SAMN23380189, SAMN2338010190, SAMN2338010191, SAMN2338010192, SAMN2338010193, SAMN2338010194); 2. Seed ITS (SAMN23401314, SAMN23401315, SAMN23401316, SAMN23401317, SAMN23401318, SAMN23401319); 3. Leaf 16s (SAMN23394854, SAMN23394855, SAMN23394856, SAMN23394857, SAMN23394858, SAMN23394859); 4. Leaf ITS (SAMN23394000, SAMN23394001, SAMN23394002, SAMN23394003, SAMN23394004, SAMN23394005).

Additional information

No additional information is available for this paper.

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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