Rhizosphere microbiome analysis of healthy and infected cumin (Cuminum cyminum L.) varieties from Gujarat, India

Dinesh Kumar a, b, Meenu Saraf b,*, Chaitanya G. Joshi a, Madhvi Joshi a, b

a Gujarat Biotechnology Research Centre (GBRC), Department of Science and Technology (DST), Government of Gujarat, 6th Floor, MS Building, Gandhinagar, Gujarat 382011, India
b Department of Microbiology and Biotechnology, School of Sciences, Gujarat University, Navrangpura, Ahmedabad, Gujarat 380009, India

ARTICLE INFO

Keywords: Rhizosphere Fungal infection Host plant pathogen Microbiome, Co-occurrence network

ABSTRACT

Cumin (Cuminum cyminum L.; Jeera) is a popular herbal seed spice used in culinary preparation in India. Gujarat and Rajasthan are the largest producer states of cumin seeds from India, while India is also the largest supplier and exporter of cumin across the globe. However, production of cumin is suffering from loss of crop production due to the plant pathogen infections, especially from Fusarium oxysporum sp. Rhizosphere microbiome is the key modulator of plant health, revitalizing nutrients and disease response against plant pathogens. The secretion of different metabolites such as root exudates plays an important role in host plant rhizosphere microbial interactions influencing the plant health, growth and development, nutrient acquisition, and disease resistance. Therefore, in this research study, we have examined the microbial diversity from the healthy and fungal infected rhizosphere samples of the three different Gujarat Cumin (GC-2, GC-3, and GC-4) varieties using 16S ribosomal RNA (rRNA) gene sequencing on Ion Torrent S5 sequencing platform. The findings revealed the major dominant family represented by Bacillaceae, Solibacteraeaceae, Nostocaceae, Paenibacillaceae, Scytonemataceae, and Halothiobacillacae, while at genera level of taxonomic abundance were represented by Bacillus, Candidatus Solibacter, Synecococcus, Nostoc, Anabaena, and Oscillatoria. The research findings should enhance our understanding of healthy and infected plant rhizosphere microbiome for better crop productivity, disease resistance and management of the crop varieties against plant pathogens.

Introduction

India has emerged as a major producer and supplier of seed spices in the world market over a period of time (Meena et al., 2018). Cumin (Cuminum cyminum L.) seeds are highly prized for the aromatic essential oils in the culinary preparation and flavouring (Choudhary et al., 2021). The majority of the cumin is produced by western Indian states, Gujarat and Rajasthan (Mishra et al., 2017; Patel et al., 2020; Sharma et al., 2018). Apart from the several other factors, the harvest of cumin is limited by mainly fungal infection caused by the root wilting by Fusarium oxysporum sp (Lodha and Mawar, 2014). The loss of crop productivity due to wilting is a challenge across the globe for various agricultural crops (Gajera et al., 2016a; Jadeja, 2008). Gujarat cumin-2 (GC-2) is a susceptible variety with 100 to 105 days of maturity period with an expected yield of 822 kg/ha, while Gujarat cumin-4 (GC-4) is resistant to wilting with better yield of 1250 kg/ha, larger seed size and volatile oil content and a maturity period of 105 to 110 days. Gujarat cumin (GC-3) is an intermediate variety with wilt resistance characteristics (Bhoraniya et al., 2017; Talaviya et al., 2017).

Rhizosphere microbiome interactions offers unique biological network that plays important role in fitness of the host plant growth and development. Similarly, the fitness trait is not singular entity to the species and needs to be broadly considered along with the microbial dark matter also known as “holobiont” supporting the plant health and fitness under highly variable environmental conditions (Hassani et al., 2018; Nobori et al., 2018; Rosenberg and Zilber-Rosenberg, 2018). The microorganisms that helps in plant growth and development through various mechanisms are also known as, Plant Growth Promoting Rhizobia (PGPR), occupies and share the same space as roots of the plants (Hartmann et al., 2009a; Rupal et al., 2020; Singh, 2006; Zhang et al., 2017). While microorganisms also involved in phosphate solubilisation and nutrient acquisition (Castrillo et al., 2017; Chinnappan et al., 2018; Choudhary et al., 2021) were isolated and identified from cumin rhizosphere. Similarly, root exudates such as organic acids, plant...
growth regulators, vitamins and amino acids are known to modulate the microbial composition. These implications of the uniqueness are not only restricted to below the ground microbiomes, but also influence the recruitment in the endosymbionts and above the ground diverse microbial community structure such as the leaf, stem and seeds (Mishra et al., 2019; Mitter et al., 2017; Verma and White, 2018; Walitang et al., 2018).

Recent advances in high throughput genomics and computational biology make is possible to understand complex microbiomes at different level of the taxonomic abundance, distinct functional profile with the specific genotypes of plant species under regulated and controlled environmental conditions (Compart et al., 2019; Kumar et al., 2021). The plants are under the constant interaction of the microbial populations in the rhizosphere. *Pseudomonas syringae* is a well-known plant pathogen having a very broad host range including tomato, tobacco, green leafy plants and beans (Coutinho et al., 2015; Talaviya and Jadeja, 2015). Another well-known pathogenic bacterium is *Erwinia amylovora* that causes fire blight disease of fruit trees and ornamentals plants. *Xanthomonas* species, *Ralstonia solanacearum* and *Xylella fastidiosa* are also associated with several important diseases of crops like potato and banana (Hartmann et al., 2009a; Singh, 2006). Recent studies revealed the molecular aspects by exploring the biocontrol activities against plant pathogen invasion and disease response through the production of antibiotics, lytic enzymes, pathogen-inhibiting volatile compounds and siderophores (Ankati et al., 2019; Hartmann et al., 2009b). Some bacteria protect the plant from pathogens by limiting plant hormones level and inducing plant systemic resistance (Lakshmanan et al., 2012). In particular, genera like *Pseudomonas*, *Streptomyces*, *Bacillus*, *Paenibacillus*, *Enterobacter*, *Pantoea*, *Burkholderia* and *Paraburkholderia* have been reported for their role in pathogen suppression (Coutinho et al., 2015; Zorner et al., 2018).

Therefore, in order to understand the rhizosphere microbial diversity of bacterial population in the healthy and fungal infected cumin plants, in this research study, we have examined the rhizosphere samples of three different Gujarat cumin (GC-2, GC-3, and GC-4) varieties using 16S ribosomal RNA (rRNA) gene sequencing. Further, understanding the role of rhizosphere microbial communities should help in improving the crop production, improvement of plant health and disease resistance.

**Material and methods**

**Sample collection**

The rhizosphere soil samples were collected from the wilt sick plot from Seed Spices Research Station (SSRS), Sardarkrushinagar Dantiwada Agricultural University, Jagudan, Banaskantha, Gujarat, India (23°30′50.0″ N, 72°23′57.0″ E). The fungal infected and healthy plants were selected from the designated fungal resistance genotype-screening field sites. Approximately, thirty-five days old plant saplings (GC-2, GC-3, and GC-4) were selected for collecting the rhizosphere samples after removing five cm of top layer of the soil in sterile containers with the help of the technical staff at wilt sick plots. The samples were transported to the laboratory and processed for the downstream processing.

**DNA isolation and next generation sequencing library preparation**

The total genomic DNA from the rhizosphere soil samples was extracted using PowerSoil DNA isolation kit (QIAGEN, Germany) as per the manufacturer’s protocol. After isolation, the assessment of DNA quality was checked on 0.8% agarose gel electrophoresis and visualized on a gel-documentation system (Bio-Rad, USA). The quantification of the DNA was done by using QIAxpert System (QIAGEN, Germany) and Qubit 4 Fluorometer (Invitrogen, USA). The V2-V3 hyper variable region of the 16S rRNA gene were amplified using polymerase chain reaction (PCR) using 101 F (5′-ACTTACGGGCTCAGTGTA3′) and 518 R (5′-CGTATTACCGCGGCTGCTG3′) universal primer set from the extracted total rhizosphere genomic DNA samples. The primers were synthesized commercially and purchased from Eurofins Genomics India Pvt., India. The quality of the genomic DNA isolated from the rhizosphere samples was checked for the PCR amplification efficiency as the standard PCR conditions for the non-barcoded 16S rRNA gene universal primer sets. Total genomic DNA in all the samples were normalized to an equimolar concentration of 5 ng/μl for PCR amplification. The PCR amplification was carried out in a total of 20 μl reaction volume containing 10 μl of EmeraldAmp® GT PCR Master Mix (Takara, Japan), 6 μl nuclease free water, 1 μl (2 mg/ml) of bovine serum albumin (BSA) (HI-Media, India), 1 μl (5pM) each for the forward and reverse primers, and 2 μl of extracted genomic DNA. The PCR cycling conditions were set at initial denaturation was set at 94 °C for 5 min, denaturation for 25 cycles at 94 °C for 60 s, annealing at 58 °C for 25 s, and extension at 72 °C for 50 s. The final extension was at 72 °C for 5 min using Veriti™ 96-Well Thermal Cycler (Applied Biosystems, Thermofisher, USA).

Purification of the PCR amplified library was done using magnetic bead based Agencourt AMPure XP kit (Beckman Coulter, USA) method as per the manufacturer’s protocol to remove small fragments <100 bp. The metagenome libraries were quantified fluorometrically on a Qubit fluorometer 4.0 (Invitrogen, USA). The emulsion PCR of pooled libraries was carried out using Ion OneTouch 2 (OT2) System (Invitrogen, USA) by template amplification and enrichment for the sequencing as per the manufacture’s protocol. The next generation sequencing was performed using Ion GeneStudio S5 Plus platform using Ion 530v1 chip and 400 bp chemistry.

**Data quality filtering and bioinformatics analysis**

The raw amplicon sequencing dataset obtained in the FASTQ format was filtered and quality checked for further downstream analysis and processing using Prinseq-Lite v0.20.4 (Schmieder and Edwards, 2011) with a minimum mean quality value of Q25. FastQC v0.11.5 (Anders and others, 2010) was used to check the quality of the filtered dataset. The obtained 16S ribosomal RNA (rRNA) gene sequencing results are summarized in Supplementary Table 1. The good quality filtered 16S rRNA gene sequencing data files were uploaded on MG-RAST version 4.0.3 (Aziz et al., 2008) server for taxonomic analysis. After quality control and filtering (removing ≥5% of the artificial sequences and ambiguous bases), sequences with the “Best hit” criteria were used for the taxonomical analysis with default parameters against the SILVA-SSU database (Quast et al., 2012). The data normalization, library size overview and rarefaction curve (Supplementary Fig. 1) analysis was performed to address the variability in sampling depth and the sparsity of the data for comparative analysis. The rarefaction curve attained the plateau signifying the taxonomic sampling depth and coverage in the sequenced metagenome samples.

**Heat tree analysis, LEfSe and co-relation network analysis**

The heat tree analysis was performed to leverages the hierarchical structure of taxonomic classifications to quantitatively using the median abundance and statistically method using the non-parametric Wilcoxon Rank Sum test to determine taxonomic differences between microbial communities for different group comparisons. The samples were grouped and analysed for the healthy and infected cumin plant rhizosphere soil samples. The core microbiome analysis was performed based on the criteria of minimal sample prevalence criteria of twenty percent and relative abundance (0.01%) while considering all samples together, independent of the experimental factor. The core microbiome and co-occurrence network analysis was performed using MicrobiomeAnalyst webserver (Chong et al., 2020). The biomarker microbial community structure analysis was performed using LEfSe (Segata et al., 2011) at the family and genus level to identify the dominant biomarker taxa in the healthy and infected cumin rhizosphere samples.
Statistical analysis

The rarefaction curve was obtained using PAST software 4.0.3 (paleontological Statistics) to measure the sample representativeness against the 16S rRNA reads from the sequencing. The estimation of diversity indices and species richness were calculated using PAST software version 4.0.3 (Hammer et al., 2001). Non-metric multidimensional scaling (NMDS) analysis was performed based on the Bray-Curtis distance measure by the permutational multivariate analysis of variance (PERMANOVA) between the healthy and infected plant rhizosphere samples. The Principal Coordinates Analysis (PCoA) plot and dendrogram clustering was performed at species level using Bray-Curtis distance measure using Ward clustering method by MicrobiomeAnalyst webserver bioinformatics analysis pipeline (Dhariwal et al., 2017). The heatmap plots were visualised in the webserver (Metsalu and Vilo, 2015). The statistical analysis for the two group Welch’s t-test significance analysis were performed using statistical analysis of taxonomic and functional profiles (STAMP) v2.1.3 (Parks et al., 2014) software for the healthy and infected cumin rhizosphere samples.

Results

Rarefaction curve and diversity indices

The primary features of the microbial diversity analysis include the rarefaction, species richness, relative abundance, composition, distribution and coverage. The rarefaction obtained in the analysis indicate the sufficient sequencing depth in dataset by reaching plateau (Supplementary Fig. 1) for all the samples under investigation based on the approximate expected operational taxonomic units (OTUs) in a sample. The microbial diversity indices indicate the maximum observed taxa in sample CRS010 (2353), GC-2 variety, while minimum taxa were observed in sample CRS017 (1766), GC-3 variety. Similarly, Shannon and Simpson diversity indices provide inference about the community composition rather than species richness or evenness (Kim et al., 2017). The Simpson index was observed maximum for the healthy cumin rhizosphere sample CRS017 (2353), GC-2 variety, while minimum taxa were observed in sample CRS017 (1766), GC-3 variety. Similarly, Shannon index for the GC-4 healthy and infected cumin plant rhizosphere samples (p-value: 0.04512; [T-test] statistic: 3.5937) was found to be higher for the GC-4 healthy group.

Fisher index analysis was performed using one way T-test/ANOVA (p-value: 0.5401; statistic: 0.62876; while, Simpson Index (p-value: 0.32231; [T-test] statistic: 1.0214) and Shannon Index (p-value: 0.35424; [T-test] statistic: 0.95515) were analysed at the species level. The Chao1 Index (p-value: 0.64884; [T-test] statistic: 0.46563) was performed using one-way T-test ANOVA at the species level. The diversity indices analysis revealed the variation and shift in the microbial community composition and structure. To further understand the compositional difference in the microbial community structure of healthy and infected cumin plant rhizo-microbiome, Non-metric multidimensional scaling (NMDS) analysis based (Fig. 2), on the Bray-Curtis distance measure at species level was performed using PERMANOVA F-value: 1.3318; R-squared: 0.076844; p-value < 0.163 [NMDS] Stress = 0.1651 for the bacterial community composition analysis. To visualize the dissimilarity matrix, PCoA [PERMANOVA] F-value: 1.3318; R-squared: 0.076844; p-value < 0.159 was performed based on the Bray-Curtis index distance method at species level of taxonomic abundance using PERMANOVA. Furthermore, the dendrogram clustering analysis was performed using Bray-Curtis distance measure using ward clustering algorithm (Supplementary Fig. 2), while PCoA based on the Bray-Curtis distance measure with PERMANOVA statistical method at species level for the healthy and infected groups amongst the three cumin (GC-2, GC-3 and GC-4) varieties (Fig. 3).

![Fig. 1. Microbial diversity indices representing Chao1, Shannon, Fisher and Simpson index for the healthy and infected cumin rhizosphere plant samples of three different varieties (GC-2, GC-3 and GC-4).](image-url)
Rhizosphere microbiome of the healthy and infected cumin plant

The taxonomic analysis of the cumin rhizosphere soil samples revealed overall dominance of the unclassified sequences derived from bacteria in all samples with the GC-3 healthy rhizosphere sample CRS014 representing the maximum relative abundance of 42.91% while sample CRS020 was having lowest relative abundance of 24.59% at the phylum level. Similarly, next abundant phylum was represented by Firmicutes with the sample GC-4 infected cumin rhizosphere sample CRS024 representing a 40.02% while GC-3 healthy cumin rhizosphere sample CRS014 representing a proportion of 17.40%. Similarly, the Cyanobacteria, Proteobacteria, and Acidobacteria represented dominant phylum. Apart from unclassified (derived from bacteria), Firmicutes, Cyanobacteria, Proteobacteria, Acidobacteria, and Actinobacteria were found dominant at the phylum level, while Bacilli, Solibacteres, Gammaproteobacteria, Betaproteobacteria, and Clostridia were dominant at the class level of taxonomic abundance. The heatmap plot of relative taxonomic abundance at the order level is provided in Supplementary Fig. 3, while Supplementary Table 3 provide the relative abundance of the identified microorganisms at the different taxonomic levels in the three different cumin varieties (GC-2, GC-3, and GC-4) rhizosphere samples of healthy and infected cumin plants. The statistical analysis performed using STAMP v2.1.3 (Parks et al., 2014) revealed the Firmicutes, Proteobacteria, Acidobacteria, and Actinobacteria as the dominant phylum while Bacillaceae, Nostocaceae, Solibacteraceae, Scytonemataceae, Paenibacillaceae, and Burkholderiaceae were found to be dominant at the family level of taxonomic abundance.

Microbial diversity and taxonomic abundance at phylum level

The dominance of the microbial taxa at phylum level taxonomic abundance of the Gujarlat cumin-2 (GC-2) rhizosphere samples revealed the dominance of the unclassified sequences derived from bacteria (36%), Firmicutes (22%), Proteobacteria (15%), Acidobacteria (12%), Cyanobacteria (9%), Actinobacteria (3%), and Bacteroidetes (1%) in sample CRS007, representing the healthy Gujarat cumin (GC-2) variety. Similarly, unclassified sequences derived from bacteria (30%), Cyanobacteria (21%), Firmicutes (18%), Proteobacteria (13%), Acidobacteria (12%), Actinobacteria (2%), and Bacteroidetes (1%) in sample CRS010, representing the infected GC-2 variety rhizosphere sample. Similarly, the microbial diversity analysis at phylum level of the Gujarat Cumin (GC-3) variety revealed the abundance of Firmicutes (39%), Cyanobacteria (12%), Proteobacteria (10%), Acidobacteria (7%), Actinobacteria (4%), Bacteroidetes (1%), and Aquificae (1%) were dominant in sample

Fig. 2. Non-metric multidimensional scaling (NMDS) analysis at the species level of taxonomic abundance (a) Healthy and infected cumin rhizosphere samples, and (b) Gujarat cumin (GC-2, GC-3, and GC-4) varieties.

Fig. 3. Principal Coordinate Analysis (PCoA) based on the Bray-Curtis distance measure with the permutational manova (PERMANOVA) statistical method at species level (a) PCoA for the healthy and infected group, and (b) GC-2, GC-3, and GC-4 varieties.
The taxonomic analysis of the Gujarat cumin-4 (GC-4) at the phylum level revealed the dominance of Firmicutes (33%), Cyanobacteria (14%), Proteobacteria (13%), Acidobacteria (7%), Actinobacteria (4%), and Bacteroidetes (1%) in sample CRS017.

The taxonomic analysis of the Gujarat cumin-4 (GC-4) at the phylum level revealed the dominance of Firmicutes (33%), Cyanobacteria (14%), Proteobacteria (13%), Acidobacteria (7%), Actinobacteria (4%), and Bacteroidetes (1%) were found to be dominant in sample CRS021, representing healthy GC-4 variety. While, microbial diversity analysis revealed Firmicutes (28%), Unclassified sequences derived from bacteria (26%), Cyanobacteria (18%), Proteobacteria (14%), Acidobacteria (8%), Actinobacteria (4%), and Bacteroidetes (1%) were dominant in GC-4 infected plant rhizosphere soil sample CRS024. The heatmap plot of relative abundance at the phylum level (Fig. 4), for the healthy and infected experimental groups representing three different Gujarat cumin (GC-2, GC-3, and GC-4) varieties revealed overall taxonomic abundance. Overall, significant phylum in the GC-2 cumin rhizosphere samples were represented by Firmicutes (p-value: 0.04778) with the mean relative abundance of 0.13% and 0.19% in the healthy and infected groups, respectively. The rhizosphere samples in the GC-3 variety were represented by Firmicutes, Bacillaceae, Solibacteraceae, and others (22%) in sample CRS007. Further, isolation and screening of the potential PGPR isolates could help in designing microbial consortium for improvement in crop yield and productivity (Yanti et al., 2021). Even, though relative abundance of some of the taxa at family level such as Aeromonadaceae, Segniliparaceae, Bradyrhizobiaceae, and Ruminococcaceae level was found to be less than one percent, however, were associated with healthy GC-2 cumin rhizosphere samples (p-value < 0.02). It would be interesting to further understand the role of dominant taxa or would be interesting to further understand the role of dominant taxa or

Microbial diversity and taxonomic abundance at family level

The taxonomic analysis at the family level of the Gujarat cumin (GC-2) variety revealed the dominance of Bacillaceae (16%), Solibacteraceae (12%), Nostocaceae (3%), Halothiobacillaceae (2%), and Paenibacillaceae (2%) and others (21%) in sample CRS007. Further, Bacillaceae (14%), Solibacteraceae (12%), Nostocaceae (5%), Halothiobacillaceae (2%), and Paenibacillaceae (1%) were found dominant in sample CRS011 representing infected GC-2 plant rhizosphere soil sample. Similarly, the microbial diversity analysis at family level of the Gujarat Cumin (GC-3) variety revealed the abundance of Bacillaceae (31%), Solibacteraceae (6%), Nostocaceae (4%), and Paenibacillaceae (2%) in sample CRS013. While Bacillaceae (19%), Nostocaceae (10%), Solibacteraceae (8%), Paenibacillaceae (1%), and Halothiobacillaceae (1%) found dominant in sample CRS018, representing infected cumin GC-3 plant. Microbial diversity analysis at the family level of the Gujarat cumin (GC-4) variety highlighted the dominance of Bacillaceae (26%), Solibacteraceae (8%), Nostocaceae (5%), Paenibacillaceae (2%), and Halothiobacillaceae (1%) in sample CRS019 representing healthy GC-4 variety. Furthermore, Bacillaceae (25%), Others (22%), Solibacteraceae (8%), Nostocaceae (5%), Paenibacillaceae (3%), and Halothiobacillaceae (1%) in sample CRS023, representing infected GC-4 cumin plant rhizosphere soil. Overall, Bacillaceae, Solibacteraceae, and Nostocaceae were dominant at family level of taxonomic classification. Further, isolation and screening of the potential PGPR isolates could help in designing microbial consortium for improvement in crop yield and productivity (Yanti et al., 2021). Even, though relative abundance of some of the taxa at family level such as Aeromonadaceae, Segniliparaceae, Bradyrhizobiaceae, and Ruminococcaceae level was found to be less than one percent, however, were associated with healthy GC-2 cumin rhizosphere samples (p-value < 0.02). It would be interesting to further understand the role of dominant taxa or

Fig. 4. Heatmap of relative abundance at the phylum level in healthy and infected group of the rhizosphere soil samples amongst three different (GC-2, GC-3, and GC-4) cumin varieties.
the rare taxa that might influence functional aspects such as disease susceptibility outcomes and hence could play important role in plant growth, development and productivity.

**Heat tree, co-occurrence network and core microbiome analysis**

The compositional taxonomic profile analysed by the heat tree analysis revealed the relational community structure of healthy and infected cumin rhizosphere samples for the GC-2, GC-3 and GC-4 varieties (Fig. 5). The observed phyla were represented by the Proteobacteria, Firmicutes, Actinobacteria and Cyanobacteria communities. The analysed experimental samples within the same order, the similar taxa were clustered together on the same root branches based on the median abundance reflecting the structure and composition of the microbial diversity. Overall, heat tree analysis indicating the compositional taxonomic abundance analysed at the order level for the comparison groups amongst the healthy and infected rhizosphere microbiome samples. The healthy cumin plant rhizosphere group were characterised by Gallionellales and Oscillatoriales as the dominant taxonomic order based on Wilcoxon rank sum test, and median log2 ratio with p-value: 0.05. Similarly, the infected cumin plant rhizosphere samples were dominated by Entomoplasmatales, Nitrosonomadales, Acidobacteria, Clostridia and Rhodospirillales with p-value < 0.04. The correlations between the occurrence of the rhizobacterial genera were calculated using the SparCC correlation algorithm (Permutation SparCC: 100; P-value: 0.05, Correlation threshold: 0.6 by mean abundance, and Healthy/Infected MD-index: 0.0361) followed by the graphical inference of the network and the estimation of co-occurrence network topological properties at the family level of the taxonomic abundance (Fig. 6a).

The core microbiome genera included uncultured bacterium, Solibacter usitatus, Alpha and Delta-proteobacterium, followed by Bacillus niacin, Bacillus megaterium, Oscillatoria spongialis, Nostoc muscorum and Nodularia spumigena (Fig. 6b). The correlation analysis at the phylum level are represented in Fig. 7, highlighting the correlated phyla in healthy and infected rhizosphere soil samples. The Nitrospirae (Correlation coefficient: 0.48022, p-value: 0.04369) was found to be positively co-related to the infected rhizosphere samples compared to the healthy group at phylum level.

**Biomarker analysis using linear discriminant analysis effect size (LEfSe)**

Apart from the healthy and infected groups, different cumin varieties (GC-2, GC-3, and GC-4). The family level biomarker taxa were represented by Nostocaceae, Gallionellaceae, Burkholderiaceae, Prochlororhizaceae, and Methylcoccales in the healthy cumin rhizosphere group. While, Cylindropermum sp, Sideroxydans lithotrophicus, Syndyca sp, Synechococcus sp, Cupriavidus sp, Microcystis aeruginosa, Pleurocapsa minor, and Anabaena sp. were dominant in healthy group. The biomarker at the family level were represented by Alcaligenaceae, Nocardiopsaceae, Rhodothermaceae, Nitrospiraceae, Comamonadaceae, and Clostridiaceae in the infected group rhizosphere samples as represented in the Fig. 8. However, the inherent limitation of the 16S rRNA amplicon sequencing compared to the full-length gene sequence remains a challenge for the scientific fraternity to characterize the taxonomic resolutions at the species or strain level (Poretsky et al., 2014). Therefore, it would be interesting to complement next generation sequencing approaches with culture-based methods to identify the functional role of the biomarker taxa at the species level.

**Discussion**

Fungal plant pathogens have severe impact on production of the crop and are cause of serious concern to meet the rising food demand across the globe. In this research study, we hypothesised the role of the healthy microbiota of healthy and fungal infected cumin plants the three different varieties (GC-2, GC-3, and GC-4) from the rhizosphere soil. The dominant phyla identified with the Cyanobacteria, Firmicutes, Proteobacteria, Acidobacteria, and Actinobacteria. The biochemical and molecular mechanism of Cyanobacteria needs to be further investigated in order to understand their functional behaviour against the fungal pathogens such as Fusarium. However, from the literature review, in a research study, Authors have revealed the plant growth promoting and

---

Fig. 5. Heat tree analysis and hierarchical structure of taxonomic classifications to quantitatively (using median abundance) and statistically (using non-parametric Wilcoxon Rank Sum test) depict taxonomic differences between microbial communities. The colour gradient and the size of node, edge, and label are based on the log2 ratio of median abundance at the order level. Blue and red indicate that corresponding taxa are lower and higher, respectively.
protection against Fusarium wilt by Cyanobacterial strains (Anabaena variabilis RPAN59, Anabaena laxa RPAN8) under controlled environmental conditions in tomato (Das et al., 2021). Further, author revealed reduced disease severity while cyanobacterial colonization positively correlated with reduced fungal population indicating the potential bioformulations for the disease management and improved productivity (Yanti et al., 2021). These results showed that a significant portion of the core microbiome composed of bacterial genera with nitrogen-fixing capabilities, disease protective and plant growth promoting rhizobia. Furthermore, core microbiome analysis was also performed for each group having distinct Gujarat cumin varieties. The presence of Alpha- and Deltaproteobacterium was observed in the individual experimental groups were abundant in the healthy rhizo-microbiome samples.

Overall, dominance of Solibacter usitatus is represented in majority of the samples, it is a member of the Acidobacteria, a group of organisms commonly found in many different soil types and sediments. The functional role of Solibacter usitatus is need to be further understood for the beneficial against the fungal infections. The metabolic and functional role of Acidobacteria phylum, which is one of the most widespread and abundant taxa in different soil and terrestrial ecosystems remains surprisingly underexplored. Furthermore, research studies identified three keystone bacterial taxa belonging to Acidobacteria, Actinobacteria, and Firmicutes that controlled the invasion of Fusarium wilt at a continental scale. The authors reported disease-suppressive ability of P. graminis PH1 against fungal root pathogen and linked soil suppressiveness with the synthesis of sulfurous volatile compounds such as dimethyl sulfoxide reductase and cysteine desulfurase (Carrión et al., 2018). Similarly, authors reported the role of endosphere bacterial community on disease (Gaeumannomyces graminis) suppression and they identified endophytes belonging to Serratia and Enterobacter as most promising candidates against Gaeumannomyces graminis (Gaiero et al., 2013; Rosenblueth and Martínez-Romero, 2006). Rhizosphere is the key modulator of plant health, revitalizing nutrients and disease response against plant pathogens. The secretion of different metabolites such as root exudates plays an important role in host plant rhizosphere microbial interactions influencing the plant health, growth and development, nutrient acquisition, and disease resistance (Chaparro et al., 2014; Pantigoso et al., 2022).

The role of the Bacillus sp. and Pseudomonas sp. isolated from the rhizosphere samples indicate their potential role as plant growth
promoting potential (Gajera et al., 2016b). These microbial communities form the dominant group in a habitat and are reported to be involved in the first step of destruction of biologically complex molecules produced by autotrophic microorganisms (Chaparro et al., 2014; Kalam et al., 2020). Microorganisms facilitating the shared interactions involved to confer the genomic attributes for the mutual interactions, host plant microbiome and nutrient cycling process. The relative abundance at the genus level is provided in Supplementary Fig. 4. Metagenomic investigation of soil rhizosphere microbial diversity from the healthy and infected plants revealed structure and dynamics of the inhibiting bacterial communities. These interactions not only help the mutual survival but also in nutrient acquisition, disease resistance, plant growth and development by the beneficial microbiome (Huang et al., 2014; Mendes et al., 2013, 2011).

The core microbiome analysis of the infected group samples indicated the presence of the uncultured bacterium, Solibacter usitatus, Bacillus niacini and Scytonema hofmanni. Furthermore, co-occurrence network analyses were performed to assess the complexity of the interactions between the microbial genera detected in the rhizosphere of healthy samples and infected plant rhizosphere microbiome. The abundance of the reads obtained from uncultured microbes derived from bacteria is relatively higher in all the samples. The correlation network analysis is a useful method to identify potential interactions between microbes that could represent mutualistic, commensal, parasitic or even competitive relationships (Phour et al., 2020). Uncovering such interactions could hold important implications for the microbial community structure and microbiome function. The members of the core microbiome shared by all eighteen rhizosphere soil samples were in higher abundance.

Host associated rhizosphere microbiome forms complex network and mutual relationship that confer beneficial traits and help in trigger of host plant defence mechanisms against the plant pathogens, adaptability and stability (Stengel et al., 2022). The microbial interactions form key component in addressing the sustainable and eco-friendly agricultural practices. Metagenomics and culture-independent methods have greatly improved our understanding of the cryptic microbial communities; biosynthetic gene clusters (BGCs) and their potential functional role in plant health, growth and promotion with the advancement in high throughput genome sequencing approaches (Herms et al., 2022; Song et al., 2021). The microbiome of the healthy plants is great perspective for improvement of the increased productivity, climate resilient agriculture practices in semi-arid and arid zones and less dependence on the externally supplied nutrients. Therefore, our research study highlights the potential microbial population dominant in the rhizosphere that should be useful genomic resource for understanding the complex host plant microbiome interactions for the better agricultural practices and global food security measures.

Conclusions

Beneficial impact of healthy soil microbiome are widely recognized as the modulators of plant health, nutrient acquisition, disease resistance and productivity. Our research study highlights the rhizosphere microbiome assessment and microbial community structure analysis from the healthy and fungal infected Gujarat cumin (GC-2, GC-3, and GC-4) varieties. These findings revealed the dominant marker taxa in the healthy and infected rhizosphere soil samples from the different cumin varieties. Furthermore, understanding the functional diversity of the microbial communities in healthy and infected cumin varieties should help in improvement of the crop productivity, disease resistance and soil plant microbiome interactions.

Data availability

The raw reads generated via sequencing of the rhizosphere samples has been submitted to the National Centre for Biotechnology Information (NCBI) under Sequence Read Archive (SRA) under the Bio-project accession number PRJNA772370 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA772370) with Bio-sample accession numbers SAMN22377356 to SAMN22377373.

Ethics approval

This article does not contain any studies with human participants or animals performed by any of the authors.
GC-3, and GC-4).

sick plots of the different cumin varieties (Gujarat cumin varieties GC-2, for help and support in the rhizosphere sample collection from the wilt
Resources
erman of Gujarat, Gandhinagar, Gujarat for providing the infrastruc
view
Resources

Supplementary Table 2: Taxonomic abundance table of the Gujarati cumin (GC-2, GC-3 and GC-4) samples.

Supplementary Fig. 4: Taxonomic bar plot of the relative abundance at the genus level for the healthy and infected cumin rhizosphere (GC-2, GC-3 and GC-4) samples.

Supplementary Table 1: Raw reads and quality filtered reads post processing from the Cumin rhizosphere samples with the minimum quality mean score 25 using Prinseq-Lite v0.20.4.

Supplementary Table 2: Diversity indices of the rhizosphere samples of the healthy and infected Cumin varieties (GC-2, GC-3 and GC-3).

Supplementary Table 3: Taxonomic abundance table of the Gujarati cumin (GC2, GC-3, and GC-4) varieties.

CRediT authorship contribution statement

Dinesh Kumar: Data curation, Investigation, Formal analysis, Writing – review & editing. Meenu Saraf: Conceptualization, Investigation, Formal analysis, Writing – review & editing, Writing – original draft. Chaitanya G Joshi: Conceptualization, Investigation, Formal analysis, Writing – review & editing, Writing – original draft.

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.

Acknowledgement

Authors would like to acknowledge the Gujarat State Biotechnology Mission (GSBTM), Department of Science and Technology (DST), Government of Gujarat, Gandhinagar, Gujarat for providing the infrastructure support and research facility. Seed Spices Research Station (SSRS), Sardarkrushinagar Dantiwada Agricultural University, Jagudan, Gujarat for help and support in the rhizosphere sample collection from the wilt sick plots of the different cumin varieties (Gujarat cumin varieties GC-2, GC-3, and GC-4).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cmicr.2022.100163.
Lakshmanan, V., Kitto, J.L., Hsu, Y.H., Kearns, D.B., Wu, Y.S., Batz, H.P., 2012. Microbe-associated molecular pattern-triggered root responses mediate beneficial Rhizobacterial recruitment in Arabidopsis. Plant Physiol. 160, 1643–1661. https://doi.org/10.1104/pp.111.200386.

Lodha, S., Mawar, R., 2014. Cumin wilt management – a review 23, 145–155.

Meena, M.D., Lal, G., Meena, S.S., Meena, N.K., 2018. Production and export performances of major seed spices in India during pre and post-WTO period. Int. J. Seed Spices 8, 21–30.

Mendes, R., Garbeva, P., Raaijmakers, J.M., 2013. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol. Rev. 37, 634–663. https://doi.org/10.1111/1574-6976.12028.

Metsalu, T., Vilo, J., 2015. ClustVis: a web tool for visualizing clustering of multivariate data using principal component analysis and heatmap. Web Serv. 43 https://doi.org/10.1093/nar/gkv468 issue Publ. online.

Mishra, B.K., Dubey, P.N., Sharma, Y.K., Kant, K., 2017. Rhizosphere effect in seed spices plants grown under semi-arid conditions. Int. J. Seed.

Mishra, B.K., Lal, G., Sharma, Y.K., Kant, K., Saxena, S.N., Dubey, P.N., 2019. Effect of microbial inoculants on cumin (Cuminum cyminum Linn.) growth and yield. Int. J. Seed Spices 53, 53–56.

Mitter, B., Pfaffenbichler, N., Flavell, R., Compant, S., Antonielli, L., Petric, A., Berninger, T., Naveed, M., Shethi-Tezerji, R., von Maltzahn, G., 2017. A new approach to modify plant microorganisms and traits by introducing beneficial bacteria at flowering into progeny seeds. Front. Microbiol. 8, 11.

Neborl, T., Mine, A., Tsuda, K., 2018. Molecular networks in plant–pathogen holobiont. FERS Lett.

Pantigoso, H.A., Newberger, D., Vivanco, J.M., 2022. The rhizosphere microbiome: plant-microbe interactions for resource acquisition. J. Appl. Microbiol. 1–13. https://doi.org/10.1111/jam.15686.

Parks, D.H., Tyson, G.W., Hugenholtz, P., Beiko, R.G., 2014. STAMP: statistical analysis of functional and taxonomic profiles. Bioinformatics 30, 3123–3124. https://doi.org/10.1093/bioinformatics/btu494.

Patel, A., Tiwari, S., Pandey, N., Gupta, D., 2020. Role of spices beyond a flavouring agent: the antioxidant and medicinal properties. ... Investig. Indian.

Phour, M., Sehrawat, A., Sandhu, S.S., Glick, B.R., 2020. Interkingdom signaling in plant-rhizomicrobiome interactions for sustainable agriculture. Microbiol. Res. https://doi.org/10.1016/j.micres.2020.126589.

Poretsky, R., Rodríguez-R, I.M., Luo, C., Tsiemenski, D., Konstantinidis, K.T., 2014. Strengths and limitations of 16S rRNA gene amplicon sequencing in revealing temporal microbial community dynamics. PLoS One 9. https://doi.org/10.1371/journal.pone.0093827.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glockner, F.O., 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596.

Rosenberg, E., Zilber-Rosenberg, I., 2018. The hologenome concept of evolution after 10 years. Microbiome 6, 78.

Rosenbluh, M., Martínez-Romero, E., 2006. Bacterial endophytes and their interactions with hosts. Mol. Plant-Microbe Interact. 19, 827–837.

Rupal, K.S., Raval, V.H., Saraf, M., 2020. Biosynthesis and purification of indole-3-acetic acid by halotolerant rhizobacteria isolated from Little Rann of Kachchh. Biocatal. Agric. Biotechnol. 23, 101435 https://doi.org/10.1016/j.bcab.2019.101435.

Schneider, R., Edwards, R., 2011. Quality control and preprocessing of metagenomic datasets. Bioinformatics 27, 863–864. https://doi.org/10.1093/bioinformatics/btr026.

Selega, N., Izzard, J., Waldron, L., Gevers, D., Miyazaki-Pacheco, Y., Garrett, W.S., Huttenhower, C., 2011. Metagenomic biomarker discovery and explanation. Genome Biol. 12, 1–18.

Sharma, S.S., Rao, S.S., Singh, R.S., Sharma, R.P., Dubey, P.N., 2018. Evaluation of potential cumin growing area in hot arid region of Jaisalmer district 8, 50–55.

Singh, S.K., 2006. Effect of plant growth promoting rhizobacteria on potato. Potato J. 33, 164–165.

Song, S., Liu, Y., Wang, N.R., Haney, C.H., 2021. Mechanisms in plant-microbiome interactions: lessons from model systems. Curr. Opin. Plant Biol. 62, 102003.

Stengel, A., Driiber, R.A., Carr, E., Egeja, T., Hillman, E., Krause, T., Renee, S., Herr, J.R., 2022. Rethinking the roles of pathogens and mutualists: exploring the continuum of symbiosis in the context of microbial ecology and evolution. Phytobiomes 6, 108.

Talaviya, J.R., Jadeja, K.B., 2015. Efficacy of Bioagents alone and in combination varieties/lines against wilt disease. Int. J. Curr. Microbiol. Appl. Sci. 6, 3176–3177. https://doi.org/10.20546/ijcmas.2017.606.373.

Verma, S.K., White, J.F., 2018. Indigenous endophytic seed bacteria promote seedling development and defend against fungal disease in brownspot millet (Urochloa ramosa L.). J. Appl. Microbiol. 124, 764–776.

Wallant, D.J., Kim, C.G., Kim, K., Kang, Y., Kim, Y.K., Sa, T., 2018. The influence of host genotype and salt stress on the seed endophytic community of salt-sensitive and salt-tolerant rice cultivars. BMC Plant Biol. 18, 51.

Yanti, Y., Hamid, H., 2021. Development of the PGPR and cyanobacteria consortium for development and defend against fungal disease in browntop millet (Urochloa ramosa L.). J. Appl. Microbiol. 124, 764–776.

Zorner, P., Farmer, S., Alibek, K., 2018. Quantifying crop rhizosphere microbiome temporal microbial community dynamics. PLoS One 9. https://doi.org/10.1371/journal.pone.0093827.