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Alteration of Rice Root Endophytic Bacterial Community Composition by *Meloidogyne graminicola* and Identification of Potential Biocontrol Agent

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Abstract

Introduction Rice root gall is a severe infection caused by the rice root-knot nematode *Meloidogyne graminicola*. Overuse of chemical nematicides intensifies the need for a suitable biocontrol agent. Nematode infestation in plants alters the associated microbiome; however, their correlations need to be better understood. Hence, this work aimed to unravel the changes in indigenous endophytic bacterial community composition of rice root because of infection caused by *M. graminicola* and also to identify dominant bacteria strains as a potential biological control agent.

Material & Methods The endophytic bacterial community of non-infected rice root and gall was analysed using a 16 S rRNA gene-based metagenomics approach. The dominant endophytic bacterial community was further isolated and screened for its PGP and nematicidal activity using bacterial cell suspension and culture filtrate to identify a potential biocontrol agent.

Result and Discussion Our results show that nematode infection has altered the bacterial community composition, and a distinct community existed between gall and non-infected roots. This shift in the microbial community is associated with reduced species richness due to infection. We also observed that a few endophytic genera like *Chryseobacterium*, *Rhizobium*, *Gemmata*, and *Pseudomonas* that were unique to gall are reported to have been associated either with nematode or may have been recruited by plants as a growth promoter to combat nematode infection. Other bacterial endophytes that are specific to the non-infected root microbiome, like *Delftia*, *Bacillus*, *Pantoea*, *Acidovorax*, and *Azorhizobium*, are hypothesised to remain associated with rice seeds, and they possess biological control/plant growth promotion abilities. Further, after screening all isolates, *Enterobacter* sp. strain SSNI 8 isolated from a non-infected root was evaluated for its efficiency in acting as a nematicidal agent against *M. graminicola*, and we found that the strain showed 90% nematode mortality with its culture filtrate which may possess some secondary metabolites antagonistic to the nematode.

Conclusion Overall, this study provided a comprehensive view of endophytes associated with gall in non-infected roots and identified a potential biocontrol agent.

Keywords Metagenomics, Rice root gall, Plant growth promoters, Nematicidal activity, *Meloidogyne graminicola*

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Introduction

The rice root-knot nematode, *M. graminicola*, is particularly detrimental to irrigated and upland rice ecology in Asia, and yield loss of up to 80% has been reported under flooded conditions (Masson et al. 2020; Mondal et al. 2021). This pathogen has been declared a pest of international importance (Mondal et al. 2021). *M. graminicola* is challenging to control because of its sedentary endoparasitic nature and wide climatic adaptability. Using fumigants, various chemical nematicides and soil flooding are the principal methods to control this nematode (Kumar and Dara 2021; Mondal et al. 2021). However, the recent adoption of labour and irrigation limiting techniques and lack of availability of labelled nematicides has made the existing control methods ineffective. Thus, the necessity for an alternative approach to nematode management emerged.

In recent years, the use of microorganisms as biological control agents (BCAs) has been rising, and endophytes, in this regard, have gained particular importance (Hussain et al. 2018). Among the bacterial endophytes, the genus *Bacillus* has been mostly reported as an effective BCAs (Liu et al. 2020; Mendoza et al. 2008; Padgham and Sikora 2007), along with others, like *Pseudomonas* (Anita and Samiyappan 2012), *Klebsiella* (Liu et al. 2018) and *Enterobacter* (Zhao et al. 2022). However, using plant-associated indigenous microorganisms as BCAs would be favourable since they can quickly establish themselves in local soil, facilitating plant-microbe interactions (Kunda et al. 2020).

With the advent of culture-independent, high-throughput sequencing-based metagenomic approaches, a new era of understanding the microbiome and its potential in managing nematode infections has dawned. This promising solution against PPNs (Masson et al. 2020; Tian et al. 2015) is a beacon of hope in biological control. The association of plant microbiome with nematode infection has been elucidated in some studies (Barros et al. 2022; Deng et al. 2022; Lamelas et al. 2020; Masson et al. 2020; Tian et al. 2015) where researchers have found that nematode infestations affected the relative abundance of bacterial genera and altered core microbiome. All these research works have established that infection causes changes in microbial communities with varied potential to exist between infected and non-infected roots.

While there are several reports on *Meloidogyne* spp, only a handful of studies (Anita and Samiyappan 2012; Haque et al. 2018; Khan et al. 2021; Padgham and Sikora 2007; Seenivasan et al. 2012; Subudhi et al. 2020) on managing *M. graminicola* have been conducted in India. These reported BCAs have shown promise in managing infection, but many unknown mechanisms are still at play in biocontrol activity. Identifying microbial populations is a crucial step that requires extensive investigation to make these BCAs work under broader field conditions. This underscores the importance of our ongoing research and its potential impact on nematode control in agriculture.

In the present study, we unravelled the compositions of the bacterial communities associated with *Meloidogyne graminicola* infected galled and non-infected rice roots by culture-independent and culture-dependent approaches. Microbiome studies were conducted using an amplicon metagenomics approach. Under a culture-dependent approach, bacteria were isolated from both galled and non-infected roots to identify potential biocontrol agents against the nematode. The chosen rice variety, MTU-1010, is a prevalent rice variety susceptible to nematodes (Anila et al. 2018). There are no resistant mutant lines of the rice variety MTU 1010 against nematodes. Moreover, as ethical issues are still prevalent with genetically modified crops, these rice varieties are not produced and consumed regularly (Ambientais et al. 2002). Also, using other nematode-resistant rice varieties is not a suitable option as those varieties are not popularly cultivated and consumed. The present study evaluated an alternative, cost-effective, easy-to-use management approach to biologically control rice nematode to serve as a safe, sustainable alternative to harmful chemical pesticides and bio-fertilizers.

The objectives of this study were: (i) to investigate the endophytic microbiome of rice root infected by *M. graminicola*, (ii) to characterise the bacterial community composition associated with nematode-galled and non-infected roots, and (iii) to evaluate the role of isolated bacteria from the gall and non-infected roots as a potential biocontrol agent against the rice root-knot nematode.

Materials and methods

Nematode inoculation, sample collection and processing

The experiment was conducted during the autumn season in the month of September at the Research Farm of the Indian Statistical Institute. Rice plants, *Oryza sativa* cv. MTU 1010, a variety susceptible to root-knot nematodes, M. *graminicola* (Ravindra et al., 2015), was used for the present study and was cultivated in the Research Farm of the Indian Statistical Institute, Giridih branch. The chosen rice variety is a prevalent, high-yielding, mega variety of rice that produces long, slender grains (Anila et al. 2018). Nematodes were also obtained from the Research Farm of the Indian Statistical Institute, Giridih branch. Collected nematodes were maintained in double steam-sterilized soil on *O. sativa* cv. PB-1121 (Mondal et al. 2021). The experiment consisted of rice plants grown in twelve (12) 2000 cc plastic pots containing autoclaved soil mixed with vermicompost (2:1). Soil

parameters were: pH- 5.41, EC- 0.22, TOC- 0.87, available Phosphorus- 16.78, available Potassium- 119.93 and available Nitrogen- 54.3 and vermicompost was made with neem leaves mixed with cow dung in the ratio 1:1. Rice seeds were first surface sterilised using 90% ethanol for 2 min followed by 70% ethanol for 2 min and 4% sodium hypochlorite for 10 min. Seeds were then rinsed with distilled water a few times and placed on glass Petri plates on moist filter paper. Plates were kept inside a seed germinator for germination. After five days, fully germinated healthy seedlings of equal length were chosen, transplanted to 2000 cc pots, and allowed to grow for seven days. Afterwards, they were divided into two treatment sets, each having six pots with five seedlings in each pot. The treatments included: (a) treatment 1: nematode infected or galled set (SSI) and (b) treatment 2: non-infected or healthy set (SSNI). Treatment 1 plants were inoculated with 1000 freshly hatched J2s of *M. graminicola* near the root zone. J2s (not surface sterilized) were collected in 5 ml water and inoculated with a pipette tip near the root zone of plants at a depth of 3 cm. Plants of both treatments were maintained for 28 days in the greenhouse at the Indian Statistical Institute, Giridih. The experiment had six replications (=pot) with five plants per replicate. As samples (rice plants) were collected via the destructive sampling method, three pots from each treatment were selected for culture-independent study, and the remaining three were used for culture-dependent isolation. Post-harvest, the roots were surface sterilised using 75% ethanol and 4% sodium hypochlorite, following our previous protocol (Kunda et al. 2018). Only the galls were processed for further studies for the nematode-infected set (treatment 1).

Culture-independent studies for understanding total microbial community structure

Extraction of metagenomic DNA, amplification of 16 S rRNA gene sequences and sequence analysis

The roots of five plants per pot were taken together and then surface sterilised. The same was done for galled roots also. After surface sterilisation and sterility check, the galls were separated from the roots using a sterile scalpel. For culture-independent study, we ground the uninfected roots or galls together with liquid nitrogen and have extracted the DNA using Mo Bio Plant DNA Extraction Kit following the manufacturer's instructions. The extracted metagenomic DNA (5ng/µl) was then sent for triplicate sequencing. Sequencing was performed on the Illumina Miseq platform in a 2×300 bp paired-end run. PCR amplification of the hypervariable V3-V4 regions of bacterial 16 S rRNA gene was done with universal primers 341 F and 806R and multiplexing index sequences as well as standard adapters required for cluster generation (P5 and P7) as per the standard Illumina protocol. The amplicon libraries were prepared using the Nextera XT Index kit (Illuminainc.) and Nextera XT DNA Library Prep Kit (Part # 15044223 Rev. B). The amplicon libraries were purified by 1X AMpure XP beads and quantified using a Qubit fluorometer. The raw paired-end primer trimmed sequences were provided by Eurofins, Germany. In all the samples, the raw FastQ dataset (R1-forward read & R2-reverse read) was processed following the protocol by Kunda et al. 2021 and Dhal et al. 2020. At first, sequences were trimmed using trimmomatic v0.32 (Bolger et al. 2014). The trimmed sequences were then merged using PEAR v0.9.5 (Zhang et al. 2014) and OTU (operational taxonomic unit) clustering was performed using swarm v2.0 (Mahé et al. 2014) with default parameters. The quality-filtered OTUs were taxonomically assigned using SINA (SILVA Incremental Aligner; v1.2.11; Silva reference database release 138) (Pruesse et al. 2012) with a minimum similarity alignment of 0.9.

Isolation and characterization of the dominant culturable bacteria from the gall and non-infected rice roots

Isolation of bacteria

The surface-sterilized roots were crushed with PBS (pH-7.4), serially diluted, and plated in triplicate on NA media (Etesami and Alikhani 2016; Tashi-Oshnoei et al. 2017). All plates were incubated at 30 °C for seven days, and the emerging colonies with distinct morphologies were chosen to obtain pure cultures. Pure cultures were stored in 20% glycerol at −80 °C for further use.

Molecular identification of isolated bacteria

The genomic DNA of the pure cultures was isolated using the DNeasy UltraClean Microbial kit, a reliable and widely used method. The 16 S rRNA gene of the bacteria was then amplified using bacterial-specific universal primers, ensuring the specificity of the identification. The PCR-amplified products were gel purified and sequenced. The sequences were then compared to the NCBI database using the BLAST tool, a highly accurate and widely used sequence alignment tool. The original sequences and their three most similar sequences were aligned and a phylogenetic tree was constructed, providing a clear visual representation of the bacterial relationships. This meticulous process ensured the accuracy and reliability of the bacterial identification.

Characterization of isolates for their plant growth-promoting properties

The isolates were tested for properties that directly influence plant growth such as nitrogen fixation (Tashi-Oshnoei et al. 2017), potassium solubilisation (Hu et al.

2006), 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (Penrose and Glick 2003) and indole acetic acid (IAA) (Patten L. Cheryl and Glick R. Bernard, 2002) production. Besides, indirect (biocontrol traits) plant growth-promoting activities like the production of siderophores (Schwyn and Neilands 1987), ammonia (Sarkar et al. 2018) and hydrogen cyanide (Tashi-Oshnoei et al. 2017) were also studied following standardized protocols.

Screening of bacterial isolates for biocontrol of M. graminicola

All the isolated bacterial strains were tested for their potential (to either produce secondary metabolites or have a direct effect) in killing nematodes by following the protocol of Liu et al. 2020 and Mendoza et al. 2008 with few modifications. At first, all the bacterial isolates were cultured in BEPB (beef extract peptone broth) for 72 h at 30 °C. After 3 days, the cultures were centrifuged for 10 min at 10,062 g, and the supernatant was filtered through a 0.2 µm filter membrane (Millipore) to make cell-free extracts. The filtrate was then diluted with sterile distilled water into 2-fold dilution, and 1 ml of this filtrate was incubated with 1 ml of 100 freshly hatched J2s (in distilled water) in six-well culture plates at 28 °C for 48 h. Sterile distilled water mixed with BEPB into 2-fold dilution served as control. The number of dead nematodes was counted under a stereo zoom binocular microscope (Stemi-305, Carl Zeiss, Germany) and nematodes were considered dead when its body posture did not change after adding few drops of 1 N sodium hydroxide (NaOH) into the culture plate (Chen and Dickson 2000). All the treatments were replicated six times and the experiment was repeated twice.

To observe the effects of living bacterial cells on nematodes, bacterial cells obtained as pellets were washed with 0.9% sodium chloride (NaCl) solution and re-suspended in Ringer's solution to obtain CFU of 1×10^8. An equal volume of bacterial cell suspension was incubated with an equal volume of 100 freshly hatched J2s at 28 °C in six-well culture plates for 48 h. Ringer's solution was used as negative control. The experiment was conducted with six replicates. The number of dead nematodes was counted under a stereo zoom binocular microscope (Stemi-305, Carl Zeiss, Germany) with magnification 10X and the experiment was repeated twice.

To represent the nematicidal activity (NA) corrected mortality was calculated using Schneider–Orelli's formula, (Liu et al. 2020) where mortality (%) = number of dead nematodes/total nematodes × 100; corrected mortality (%) = (mortality % of treatments − mortality % of control)/ (100 − mortality % of control) × 100.

Dose and time dependent assay with culture filtrate of the best performing strain against M. graminicola

Among all the tested bacterial isolates, the best performing isolate in both assays was further evaluated in dose dependent and time dependent bioassays using its culture filtrate (as it had higher mortality percentage). The experiment set up had three factors: duration of incubation of the bacteria in the BEPB medium (72 h – C1 and 96 h – C2), a dosage of culture filtrate (2 times and 5 times diluted with BEPB) and incubation period of the nematodes with culture filtrate (24 h and 48 h). The isolate was cultured in BEPB for 72 h and 96 h under shaking conditions followed by centrifugation for 10 min at 10,000 rpm. The supernatant was collected and divided into two parts (treatment effect): one half was passed through a 0.2 µm filter membrane (to make it cell-free), and the other half remained as it is (not wholly cell-free with very few bacterial cells). Both parts were then diluted to two (D1) and five (D2) fold dilutions with sterile distilled water (dosage of culture filtrate). An equal volume of culture filtrates and 100 freshly hatched J2s were incubated in six healthy culture plates, and the number of dead nematodes was calculated after 24 h and 48 h (incubation period of the nematodes with culture filtrate) by the above-mentioned protocol. The experiment was repeated twice with six replications.

Statistical analysis

Culture-independent study-

The α-Diversity indices specified by OTU number, Shannon diversity index, inverse Simpson diversity index were used to measure species richness and evenness and their differences were tested with an unpaired t-test. The α-Diversity indices were measured using repeated random sub-sampling of the amplicon sequence datasets. To understand beta diversity trends in bacterial community composition between the two treatments cluster dendrogram and non-metric multidimensional scaling (NMDS) was envisaged using a Bray-Curtis dissimilarity matrix calculated on relative sequence abundance of OTUs. To obtain knowledge about the differentially abundant OTUs between the two treatment sets the sequence counts were clr-transformed with the aldex.clr function of the R package ALDEx2, using the median of 128 Monte Carlo Dirichlet instances and the result was reflected in Dotplot. All statistical analysis and figure visualization was performed in R software package, version 4.2.1 using the R core distribution (R Core Team 2022) along with additional packages vegan (Oksanen et al. 2016) and ALDE × 2 (Fernandes et al. 2014).

Culture dependent study-

For culture dependent analysis, one way analysis of variance (ANOVA) was used to identify significant differences among treatments. All the data were first checked for normality assumption and homogeneity of variance using Shapiro Wilk normality test and Bartlett test before subjected to one-way analysis of variance (ANOVA). Data were [log (x + 1)] transformed to improve normality and homogeneity of variance. The individual and interaction effects of various components of dose and time dependent assay were also analyzed by three way ANOVA (incubation time of bacteria in BEPB is one factor, dosage of culture filtrate is the other and incubation time of nematode with culture filtrate is the third) and differences were considered significant if $p < 0.01$. Significant differences between treatments sets were analyzed using Tukey HSD post hoc test. Graphs were drawn using SigmaPlot-14.0 and R software package version 4.2.1. The data are shown as the mean ± SE and data were combined if the variance was found homogenous.

Nucleotide accession number

The raw sequence metagenome data reported in this paper were submitted to NCBI with Bioproject accession numbers: PRJNA478319 and PRJNA478489. The nucleotide sequences obtained with culture dependent study were also submitted to GenBank with accession numbers OP271491-OP271520.

Results

Microbial diversity and taxonomic composition of gall and non-infected root metagenome

Amplicon sequencing of the V3-V4 region of the 16 S rRNA gene generated 28,16,383 paired end reads. Initial quality filtering resulted in 23,55,974 reads from all the samples. On average, samples from the galled set (SSI) gave rise to 372,621 sequence reads, while 412,704 sequences were obtained from the non-infected set (SSNI). After merging the paired-end reads, high-quality reads were clustered using > 97% sequence identity, creating a total of 97,326 OTUs (SSI: 48,389; SSNI: 50,889). To avoid rare biosphere and PCR artefacts, low abundance OTUs, as well as those affiliated to chloroplast and mitochondria, were removed, which resulted in taxonomically classified denoised unique sequences clustered into 5055 OTUs (SSI:3160; SSNI: 3623). The OTUs were again pruned, and finally, 3025 OTUs were obtained (Table S1). Mantel test was performed using the Bray–Curtis dissimilarities method (Mantel test, $R = 0.99$, $p = 0.001$) and the Jaccard dissimilarity method (Mantel test, $R = 0.98$, $p = 0.001$), which indicated that the trends in beta diversity were not altered after data pruning.

The Alpha diversity, i.e. within sample diversity, was indicated as rarefied average OTUs per treatment. Significant differences between the two treatment sets with respect to nOTUs were recorded by t test ($p < 0.01$). Chao 1 richness index also indicated significant difference ($p < 0.01$) between the two treatment sets. The OTUs for SSI ranged from 1484 to 1672 whereas for SSNI the range is 1864 to 1969. But no significant differences were observed for Shannon index and Inverse Simpson index (invS) (Fig S1 and Table S2).

The taxonomic composition of the gall and non-infected metagenome, as revealed at the phylum level, showed no significant differences in community composition between the groups. Among the ten most dominant phyla of SSI, *Proteobacteria* took the lead with 96% abundance, followed by *Patescibacteria* at 2%. The next abundant phyla were *Firmicutes*, *Actinobacteriota*, and *Bacteroidota*. A similar pattern was observed in SSNI, but phyla such as Planctomycetota and unclassified bacterial sequences differed significantly between the two treatments with $p < 0.05$. At the class level in SSI, the most dominant bacterial groups were affiliated with *Gammaproteobacteria* (78%), followed by *Alphaproteobacteria* (18%), *Bacteroidia* (1%), and *Saccharimonadia* (1%). This pattern was also observed in SSNI, where the abundance of the classes was *Gammaproteobacteria* (89%), *Alphaproteobacteria* (8%), followed by *Bacteroidia* and *Saccharimonadia* (1% each). The class that showed a significant difference ($p = 0.0001$) between the two treatment sets was *Planctomycetes*, which was more dominant in the non-infected set (Table S3). These differences in the taxonomic composition highlight the influence of the infection on the microbial community.

At the lower taxonomy level, the top 10 families successfully explained the variation in bacterial community composition among the two treatment sets and accounted for almost 97% of the sequences (Fig. 1). The most abundant family associated with SSI was *Oxalobacteraceae* (40%), followed by *Rhizobiaceae* (16%), *Enterobacteriaceae* (14%), *Commamonadaceae* (13%) and *Chromobacteriaceae* (8%). The pattern changed with an abundance of some families in the case of non-infected root-associated microbiome (SSNI), where *Oxalobacteraceae* was prevalent (62%), followed by *Commamonadaceae* (14%), *Enterobacteriaceae* (7%) and *Rhizobiaceae* (5%). The families that differed significantly ($p < 0.05$) between the treatments were *Enterobacteriaceae*, *Burkholderiaceae* and *Chromobacteriaceae*.

The unique and core genera (having abundance > 0.01) distributed between the two sets were identified by the Venn diagram (Fig S2). In total, eighty-eight (88)

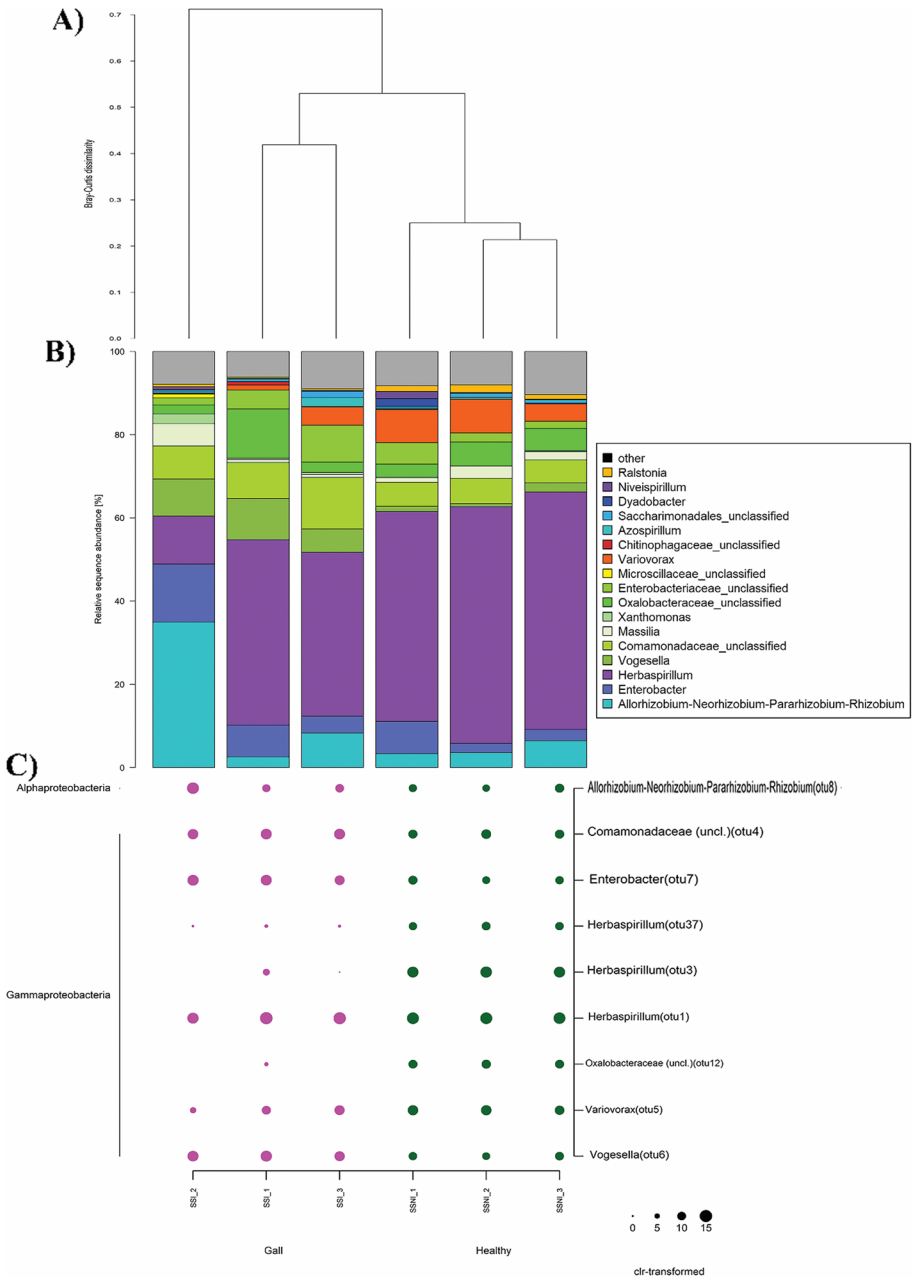

Fig. 1 The taxonomic classification of endophytic bacterial communities between gall (treatment 1) and non-infected rice root (treatment 2). A: Cluster dendrogram using Bray-Curtis dissimilarity matrix shows the distinct separation of communities in the two treatment sets at OTU level. B: Taxonomic composition of the most abundant bacterial genera per sample among the different treatment sets. C: The differentially abundant OTUs between the two treatment sets were presented in Dot Plot. Site name indicates the replica number (3) for each group: Gall (Infected) and Non-infected (Healthy). The size of each dot represents centered log ratio (clr)-transformed sequences counts. Values higher than zero indicate enrichment compared to the other OTUs per sample. The taxonomic affiliation of each OTU is provided on class (left side) and genus level (right side). Pink color represents isolate extracted from gall (infected set) and green colour represents isolates belonging to non-infected (healthy) roots

genera were common to both gall and non-infected root microbiome, and Herbaspirillum was the most prevalent, with an abundance of 32% in SSI and 55% in SSNI. However, both the sets also have few distinctive genera where gall associated microbiome possessed twenty four (24) unique genera and non-infected roots community had twenty (20) genera unique to it. Also, the abundance of the unique genera in both the sites was very low indicating they represent the rare microbiome.

Variation in microbial community composition between the two treatment sets

In contrast with the inspections made on taxonomic composition, the OTU level differences in bacterial community composition explicitly distinguished the two treatment groups and it was confirmed by cluster analysis (Fig. 1) based on Bray-Curtis dissimilarities with 0.85 average dissimilarities. This observation was also supported by NMDS ordination drawn with pruned OTUs that exhibited distinct separation of the bacterial community structure between the infected and non-infected roots (Fig S3). ANOSIM showed no significant differences between the two treatment groups. ALDEx2 was performed, and the OTUs responsible for the variation in bacterial community composition between the galled and non-infected groups were displayed in a dot plot (Fig. 1). In total, 9 differentially abundant OTUs that represented 60% and 71% coverage in galled and non-infected set respectively were identified. They were mainly affiliated to *Gammaproteobacteria* (8 OTUs) and only 1 OTU belonged to *Alphaproteobacteria*. The OTUs that were enriched in both the groups were affiliated with *Herbaspirillum* (OTU 1), *Commamonadaceae_unclassified* (OTU 4), *Variovorax* (OTU 5), *Vogesella* (OTU 6), *Enterobacter* (OTU 7) and *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* (OTU 8) whereas OTU 3, OTU 37 and OTU 12 were prevalent among the samples of the non-infected roots but were not associated with galled roots.

Isolation and molecular identification of culturable bacterial isolates

A total of thirty (30) dominant culturable bacteria were isolated from galled and non-infected roots of rice plants and their individuality was decided based on their colony morphology. Among them gall was enriched with sixteen (16) isolates while fourteen (14) isolates were hosted by non-infected roots. Molecular identification of the isolates was established by BLAST and their affiliation was confirmed by constructing phylogenetic tree with their 16Sr RNA gene (Fig. 2). The family *Enterobacteriaceae* (SSI: 25% ; SSNI : 36%) is represented by strains SSI 4, SSI 9, SSI 14, SSI 16, SSNI 1, SSNI 2, SSNI 3, SSNI 8 and SSNI 10. Of these, SSNI 2 and SSNI 10 showed more than 97% resemblance with *Enterobacter sacchari* (HQ204315). Strains SSI 14 and SSI 16 are strongly affiliated to *Enterobacter mori* (ON646184) while SSI 4 and SSNI 1 showed 59% affiliation with *Enterobacter cloacae* (HQ694001). The strain SSI 9 has 84% correlation with *Enterobacter* sp. (KX953293) whereas SSNI 8 is strongly related to *Enterobacter ludwigii* (LC015546) and SSNI 3 exhibited strong association with *Klebsiella* sp. (KJ880005). The family *Bacillaceae* (SSI: 25%; SSNI: 21%) is represented by 7 strains among which 5 strains viz. SSI 6, SSI 11, SSI 12, SSNI 5 and SSNI 11 showed strong affiliation with *Bacillus cereus* (MG778892) with bootstrap value ranging from 91 to 100% while strains SSNI 14 and SSI 10 showed 100% bootstrap affiliation with *Bacillus megaterium* (MH608333) and *Bacillus marisflavi* (KC433668) respectively. All the 3 strains (SSI 1, SSI 2, SSI 3) belonging to *Pseudomonaceae* (SSI: 19%) formed a separate clade and they were affiliated to *Pseudomonas otidis* (JQ659815) with bootstrap 73–100%. The strains SSI 15, SSNI 6 and SSNI 9 of *Erwiniaceae* (SSI: 6%; SSNI: 14%) were related to *Pantoea stewartii* (KX396015) with bootstrap value of 43–99%. Within *Comamonadaceae* (SSNI: 14%) represented by the strains SSNI 4 and SSNI 7 both showed strong correlation (100%) with *Delftia lacustris* (MG819361) and *Acidovorax temperans* (KY029032) respectively. For *Paenibacillaceae* (SSI: 13%; SSNI: 7%) strains SSI 7 and SSNI 12 showed association (74–100%) with *Brevibacillus agri* (KF957731) while strain SSI 5 formed a separate clade within the same family and was associated 100% with *Brevibacillus borstelensis* (KP279992). The strain SSNI 13 belonging to *Xanthobacteraceae* (SSNI: 7%) exhibited strong relationship with *Azorhizobium* sp. (FJ190409) with 100% bootstrap confidence. The families *Rhizobiaceae* (SSI: 6%) and *Weeksellaceae* (SSI: 6%) both represented by a single strain SSI 13 and SSI 8 had high correlation with *Rhizobium rosettiformans* (60%) and *Chryseobacterium gleum* (99%) respectively (Table 1). The NCBI-BLAST results indicate all the isolated bacteria had a strong resemblance (more than 99%) with strains isolated as endophytes or rhizospheric bacteria of rice or other plants having plant growth-promoting properties. Although members of *Comamonadaceae* and *Xanthobacteraceae* were unique to non-infected treatments, families like *Pseudomonadaceae*, *Rhizobiaceae*, and *Weeksellaceae* were gall-specific. We also made an interesting observation that the genera that were obtained in the culture-dependent study in both the groups were mostly complementary with common genera found between gall and non-infected roots in the culture-independent study, i.e. those genera that represent the dominant microbiome except for one genus, *Chryseobacterium*, which was unique to gall in both the studies.

Characterization of plant growth-promoting properties of the isolates

All the isolated strains were evaluated for their potential to possess plant growth-promoting properties, which include direct mechanisms like nitrogen fixation, potassium solubilisation, ACC deaminase and indole acetic acid production, as well as indirect mechanisms involving the formation of hydrogen cyanide, ammonia and

Fig. 2 The phylogenetic tree of the bacterial isolates based on 16 S rRNA gene sequence using neighbor- joining method and 1000 bootstraps values. Sequences represented in bold font are derived from this study. Pink color represents isolate extracted from gall (infected set) and green colour represents isolates belonging to non-infected (healthy) roots. The black colour indicates the strains with which we have drawn the similarities. Accession number for each strain is provided is parenthesis. In total, we have drawn the phylogenetic tree with our 30 isolated strains (14 from non-infected roots and 16 from gall)

siderophores (Fig. 3, Table S4). All the tested strains, except one gall-associated strain, *Brevibacillus* (SSI 5), successfully fixed atmospheric nitrogen and produced ACC deaminase. In contrast, about 44% of gall-associated isolates were potassium solubilizers belonging to genera *Enterobacter* (SSI 4, SSI 14, SSI 16), *Bacillus* (SSI 10, SSI 12), *Pseudomonas* (SSI 2) and *Kosakonia* (SSI 9) and 29% of non-infected root potassium solubilizers were affiliated with *Enterobacter* (SSNI 1, SSNI 8, SSNI 9, SSNI 10). Production of IAA was detected in all the strains of both treatments, with Bacillus (SSI 12) being the highest producer, followed by *Enterobacter* (SSI 14). Although the gall-associated isolates performed better with direct PGP properties, the trend altered with indirect mechanisms where isolates from non-infected groups showed prevalence. About 56% of gall-associated bacteria

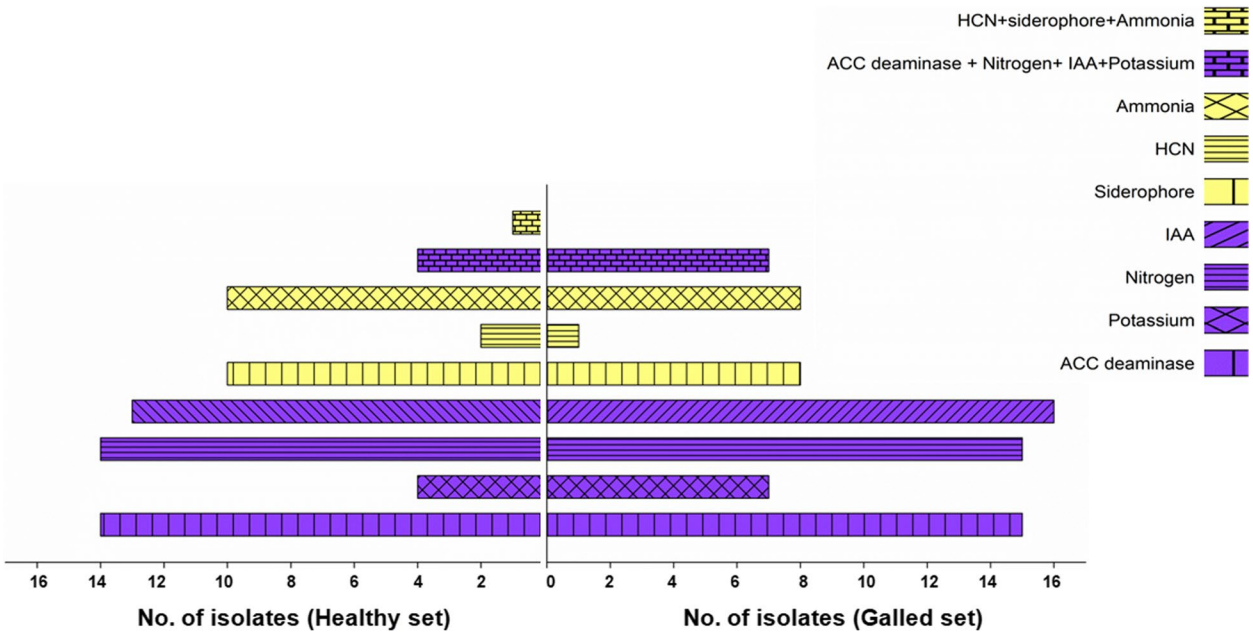

Fig. 3 Figure showing isolates that have displayed plant growth promoting (PGP) properties. Bar indicates number of isolates that have tested positive. Left side indicates non-infected set and right side belonged to galled set. The color purple is used to indicate the direct PGP properties and color yellow represented the indirect properties. Most of the isolates in the non-infected set possessed indirect plant growth promoting properties and have the ability to act as biological control agent while isolates from gall displayed direct plant growth promoting abilities

produced ammonia and siderophores mainly belonging to genus *Bacillus* (SSI 6, SSI 12), *Brevibacillus* (SSI 5, SSI 7), *Enterobacter* (SSI 4, SSI 14, SSI 16), *Kosakonia* (SSI 9), *Pseudomonas* (SSI 1, SSI 2) and *Chryseobacterium* (SSI 8), whereas 71% of non-infected root isolates viz. *Azorhizobium* (SSNI 13), *Bacillus* (SSNI 5, SSNI 11, SSNI 14), *Brevibacillus* (SSNI 12), *Enterobacter* (SSNI 1, SSNI 8, SSNI 9, SSNI 10), *Klebsiella* (SSNI 3), *Pantoea* (SSNI 6) and *Acidovorax* (SSNI 7) were capable of these properties. Very few isolates, 6% in gall and 14% in non-infected roots, were able to produce hydrogen cyanide, and they include genera like *Brevibacillus* (SSI 7), *Klebsiella* (SSNI 3) and *Acidovorax* (SSNI 7). Among all the thirty (30) isolates, none showed the potential to possess all the tested PGP properties, but one isolate of *Klebsiella* (SSNI 3) was successful in exhibiting most (6) of the properties except potassium solubilisation. The gall-associated isolates were higher in number in possessing all (4) of the direct PGP properties. Still, none of them could show all the indirect (3) properties, whereas *Klebsiella* (SSNI 3) displayed all the properties that promote plant growth indirectly.

Screening of isolates for nematicidal activity using cell-free extracts

All the isolated strains were next evaluated for their ability in producing secondary metabolites that could potentially kill J2s of *Meloidogyne graminicola*. Significant differences in corrected mortality were observed among all the isolates with Anova ($F_{29,330}$=100.4, $p<0.01$). A mortality rate greater than 50% was exhibited by four (4) strains, among which three (3) are associated with non-infected roots. The gall-associated isolate *Enterobacter mori* strain SSI 14 showed a corrected mortality of 55%. In contrast, the non-infected root isolates *Klebsiella oxytoca* strain SSNI 3, *Enterobacter* sp. strain SSNI 8, and Enterobacter sp. strain SSNI 9 exhibited corrected mortalities of 79%, 91% and 71%, respectively which are established in a bar plot (Fig S4 and Fig. 4).

Screening for nematicidal activity by cell suspension (direct killing assay)

The thirty culturable strains were also assessed for their probable role in direct nematode mortality and significant differences were observed ($F_{29,330}$=25.71, $p<0.01$) among the isolates. Three (3) gall associated strains identified as *Rosellomorea marisflavi* strain SSI 10, *Enterobacter mori* strain SSI 14 and *Enterobacter* sp. strain SSI 16 demonstrated mortality rate greater than 50% and corrected mortalities of 64%, 55% and 75% respectively. The same number of strains were also identified for the non-infected treatment where *Bacillus albus* strain SSNI 5, *Enterobacter* sp. strain SSNI 8 and *Enterobacter* sp. strain SSNI 9 displayed corrected mortalities at 53%, 82% and

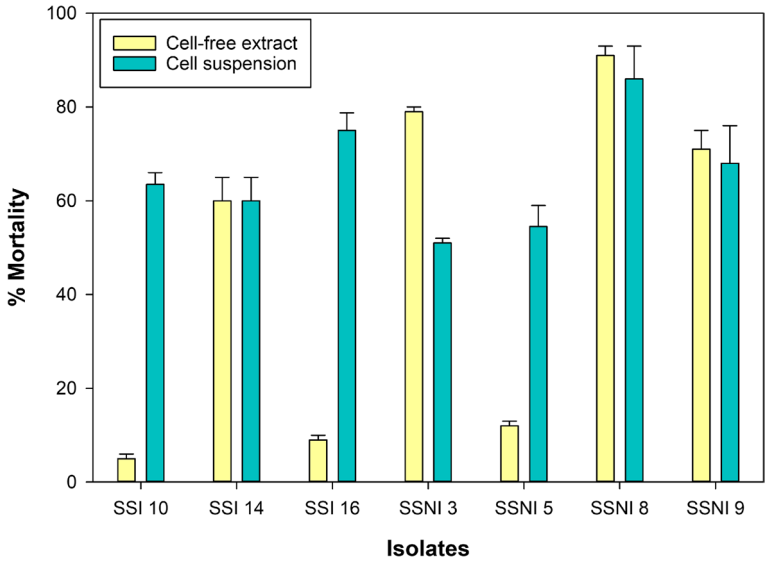

Fig. 4 Bar diagram indicating nematicidal abilities of few strains that showed corrected nematode mortality percentage more than 50. Yellow color indicates nematicidal activity with cell-free extract while green color indicates mortality of nematodes with bacterial cell-suspension. SSI indicates isolates obtained from gall (infected) and SSNI indicates bacteria isolated from non-infected (healthy) root. The data for screening of all the isolates have been shown in supplementary Fig S2

68% respectively as represented in the bar plot (Fig S4 and Fig. 4).

Dose and time dependent assay with culture filtrate of the best performing strain against M. graminicola

Finally, the isolate *Enterobacter* sp. strain SSNI 8 was selected as it could effectively kill *M. graminicola* J2s with its cell suspension and cell-free extract with a higher mortality percentage (> 80%). Hence, this isolate was further studied in a dose and time-dependent manner. In bacterial cultures grown for both C1 and C2 when both the culture filtrates were incubated with nematodes for 24 h at 2-fold dilution (C1_D1 and C2_D1), corrected mortality was about 36%, but when the same filtrate was diluted to 5-fold (C1_D2 and C2_D2) and treated with nematodes for the same time corrected mortality was reduced to 10% Again when the nematodes were incubated with the cell-free extract for 48 h in cultures grown for both C1 and C2, differences in nematicidal activity was observed in case of 2-fold dilution (D1). C1 corrected mortalities were about 86%, whereas for culture grown for C2, it stands at 90%, but for 5-fold dilutions (D2), no differences were observed where the corrected mortality was around 38% in both (Fig. 5). Studying the interaction effects among the different components revealed significant differences existed among dosage of culture filtrate ($F_{1,190}$ = 1317.726, $p < 0.001$), incubation period of bacterial culture ($F_{1,190}$ = 10.873, $p < 0.01$) and observation period of nematode mortality ($F_{1,190}$ = 1571.091, $p < 0.001$) while treatment effect had no significant variations.

Discussion

In this study, we investigated the rice root endophytic microbiome of nematode infected gall in comparison to the non-infected root by using both metagenomic and culture dependent approaches to search for any potential nematicidal agents. Both studies have confirmed the presence of a diverse bacterial community and nematode infection can influence the community composition of galled root.

Microbial community composition analysis

We observed a significant decrease in microbial richness in the gall microbiome as a result of nematode infection. A similar result was also reported by Faist et al. 2016 in infected roots of grapevine. Recently, Flemer et al. 2022 also revealed that both biotic and abiotic stress alter the endophytic microbial composition in tomato roots. They stated that stressful conditions limit photosynthetic activities in plants which alter root exudation that ultimately changes the microbial community composition of both the rhizosphere and endosphere. However, contrary to our observation, infection by root-knot nematodes has been shown to significantly enrich bacterial diversity in some studies (Masson et al. 2020; Tian et al. 2015). We hypothesize that nematode infection can increase diversity as minute punctures caused by nematode feeding allow other microbes to enter, but because

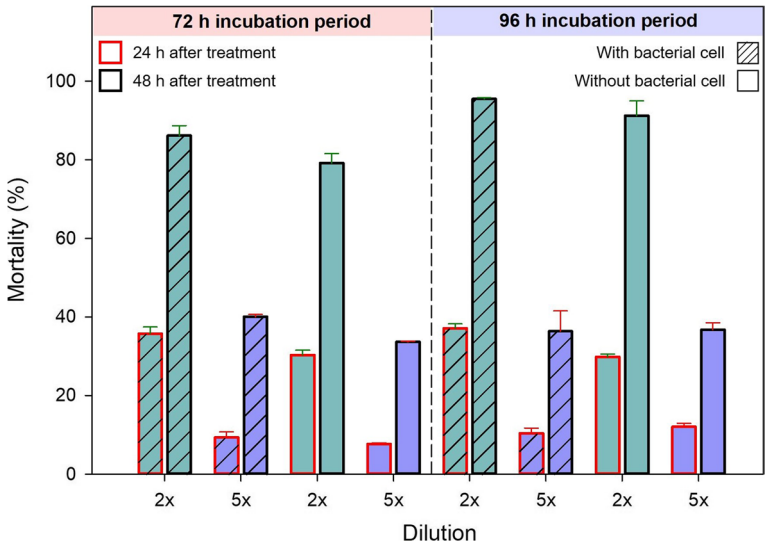

Fig. 5 Bar graph reflecting the ability of a single isolate, *Enterobacter* sp. strain SSNI 8, as nematicidal agent in a time and dose dependent study. The bar indicates corrected nematode mortality percentage. Incubation period of the bacteria was for 72 h and 96 h as given in the top of the graph. Dilution factor of culture filtrate is indicated by two colors: green color indicates twofold dilution and blue color reflects fivefold dilution. Observation duration of nematodes in culture filtrate is given by the colors red and black. Red colored outline of bar represents nematode mortality after 24 h and black colored outline shows nematode mortality after 48 h. Studying the interaction effects among the different components revealed significant differences existed among dosage of culture filtrate ($F_{1,190} = 1317.726$, $p < 0.001$), incubation period of bacterial culture ($F_{1,190} = 10.873$, $p < 0.01$) and observation period of nematode mortality ($F_{1,190} = 1571.091$, $p < 0.001$) while treatment effect had no significant variations. Significant differences between treatments sets were analyzed using Tukey HSD post hoc test. The experiment was repeated twice with six replications and the combined results are displayed

we used sterilized soil, where the microbial community was already diminished; diversity did not increase after nematode infection.

A significant alteration of the microbial community composition after infection by *M. graminicola* was also observed in our study. The lower abundance of *Planctomycetes* in roots infected with *Meloidogyne* sp. was also observed by Zhou et al. 2019 in the endosphere of eggplants and tomatoes (Zhou et al. 2019). We hypothesised that this class is associated in higher abundance with non-infected (SSNI) roots because it has been reported to be correlated with disease suppression and is also an important contributor to maintaining soil health by nitrogen cycling and nutrient turnover (Huang et al. 2015; Wang et al. 2015). Our observation regarding the enrichment of *Rhizobiaceae* in gall-associated microbiomes is also in line with Masson et al. 2020, (Cao et al. 2022; Hussain et al. 2018). Three possible explanations have been given by Wolfgang et al. 2019 for the higher abundance of this class with RKN (root-knot nematode) infection; (i) this class might be present on the nematode surface and have entered the gall during infection; (ii) manipulation of gene expressions of plant hormones and nodulation factors by RKN may increase abundance of *Rhizobiaceae* and (iii) this class has contribution in the defense mechanism of plants. Similarly, the higher abundance of *Enterobacteriaceae* in gall-associated microbiomes has also been established by Tian et al. 2015 and Faist et al. 2016 in infected tomato roots and crown galls of grapevine, respectively. Some members of this family have been found attached to the cuticle of root knot nematodes, suggesting that they entered the plant endosphere as a result of nematode infection. Members of this family can also produce plant cell-wall degrading enzymes, which may have aided in the development of infections and may be another reason for their abundance in galled roots (Tian et al. 2015). The family, *Burkholderiaceae* too followed the same pattern and was dominant in infected root as also mentioned by Lamelas et al. 2020 in studying *Meloidogyne* based disease complex in coffee and tomato plants.

The distinct separation of the two treatment sets in the OTU level also supported that microbial community composition was different between the infected and non-infected sets. Selective enrichment of OTU 3 and OTU 37, both affiliated with genus *Herbaspirillum*, and OTU 12 belonging to *Oxalobacteraceae*, in the case of non-infected roots, may be because members of this family can be involved in the suppression of soil pathogens (Cretoiu et al. 2013). Some members of *Oxalobacteraceae* are also reported to possess chitinolytic genes that degrade chitin (a component present abundantly

Table 1 Taxonomic affiliation of the 16 S rRNA gene sequence-based NCBI BLAST search results

Sl. No.	Strain ID	Isolation source	GenBank accession	BLAST affiliation (closest match)	Similarity %	Genus assigned	Species assigned	Family
1	SSI_1	Gall	OP271512	*Pseudomonas otitidis* strain R6-356 (JQ659815)	100	*Pseudomonas*	*otitidis*	Pseudomonadaceae
2	SSI_2	Gall	OP271513	*Pseudomonas otitidis* strain J11N (JX490074)	100	*Pseudomonas*	*otitidis*	Pseudomonadaceae
3	SSI_3	Gall	OP271514	*Pseudomonas otitidis* strain Tm-Ana06 (KM603640)	100	*Pseudomonas*	*otitidis*	Pseudomonadaceae
4	SSI_4	Gall	OP271507	*Enterobacter* sp. strain ERR 833 (MF442271)	100	*Enterobacter*	*sp.*	Enterobacteriaceae
5	SSI_9	Gall	OP271506	*Enterobacter ludwigii* strain PSBeC11 NRRI (MG788270)	100	*Enterobacter*	*sp.*	Enterobacteriaceae
6	SSI_5	Gall	OP271493	*Brevibacillus borstelensis* strain PGPM9 (MT150735)	100	*Brevibacillus*	*borstelensis*	Paenibacillaceae
7	SSI_6	Gall	OP271491	*Bacillus cereus* strain WG-GE-MG20 (OP093779)	100	*Bacillus*	*albus*	Bacillaceae
8	SSI_7	Gall	OP271494	*Brevibacillus agri* strain R10 (KJ206999)	100	*Brevibacillus*	*agri*	Paenibacillaceae
9	SSI_8	Gall	OP271492	*Chryseobacterium* sp. NCCP-549 (AB719399)	100	*Chryseobacterium*	*gleum*	Weeksellaceae
10	SSI_10	Gall	OP271503	*Bacillus marisflavi* strain ZJJD11 (JN999845)	100	*Bacillus*	*marisflavi*	Bacillaceae
11	SSI_11	Gall	OP271502	*Bacillus albus* strain AB-CSL3 (MG780242)	100	*Bacillus*	*albus*	Bacillaceae
12	SSI_12	Gall	OP271500	*Bacillus cereus* strain LB8 (MH187637)	100	*Bacillus*	*cereus*	Bacillaceae
13	SSI_13	Gall	OP271501	*Rhizobium rosettiformans* strain VNRL5 (KY970113)	100	*Rhizobium*	*sp.*	Rhizobiaceae
14	SSI_14	Gall	OP271516	*Enterobacter mori* strain IACMS9 (ON646184)	99	*Enterobacter*	*mori*	Enterobacteriaceae
15	SSI_15	Gall	OP271515	*Pantoea stewartii* strain AIMST Nmie4 (JF819695)	100	*Pantoea*	*stewartii*	Erwiniaceae
16	SSI_16	Gall	OP271519	*Enterobacter* sp. SC-18 (KF241160)	100	*Enterobacter*	*sp.*	Enterobacteriaceae
17	SSNI_1	Non-infected	OP271508	*Enterobacter* sp. R4-414 (JQ659708)	100	*Enterobacter*	*sp.*	Enterobacteriaceae

Table 1 (continued)

Sl. No.	Strain ID	Isolation source	GenBank accession	BLAST affiliation (closest match)	Similarity %	Genus assigned	Species assigned	Family
18	SSNI_2	Non-infected	OP271509	*Enterobacter sacchari* strain R1 (KF953912)	100	*Enterobacter*	*sp.*	Enterobacteriaceae
19	SSNI_3	Non-infected	OP271510	*Klebsiella oxytoca* strain YN201309 (KC702392)	100	*Klebsiella*	*oxytoca*	Enterobacteriaceae
20	SSNI_4	Non-infected	OP271497	*Delftia lacustris* strain AL100 (MG819361)	100	*Delftia*	*lacustris*	Comamonadaceae
21	SSNI_9	Non-infected	OP271498	*Pantoea stewartii* strain TCS5 (MK487467)	100	*Pantoea*	*stewartii*	Erwiniaceae
22	SSNI_5	Non-infected	OP271504	*Bacillus albus* strain AB-CSL3 (MG780242)	100	*Bacillus*	*albus*	Bacillaceae
23	SSNI_6	Non-infected	OP271518	*Pantoea stewartii* strain R3-124 (JQ659648)	100	*Pantoea*	*stewartii*	Erwiniaceae
24	SSNI_7	Non-infected	OP271495	*Acidovorax temperans* strain J1-5 (MG205611)	100	*Acidovorax*	*temperans*	Comamonadaceae
25	SSNI_8	Non-infected	OP271511	*Enterobacter ludwigii* strain PSBeC11 NRRI (MG788270)	100	*Enterobacter*	*sp.*	Enterobacteriaceae
26	SSNI_10	Non-infected	OP271517	*Enterobacter sacchari* strain R1 (KF953912)	100	*Enterobacter*	*sp.*	Enterobacteriaceae
27	SSNI_11	Non-infected	OP271499	*Bacillus cereus* strain AB-S2 (MG780244)	100	*Bacillus*	*cereus*	Bacillaceae
28	SSNI_12	Non-infected	OP271496	*Brevibacillus agri* strain RK25f (MG063240)	100	*Brevibacillus*	*agri*	Paenibacillaceae
29	SSNI_13	Non-infected	OP271505	*Azorhizobium caulinodans* strain SRRNI55 (KF724030)	100	*Azorhizobium*	*caulinodans*	Xanthobacteraceae
30	SSNI_14	Non-infected	OP271520	*Priestia megaterium* strain SKA-B13 (MH608333)	100	*Bacillus*	*megaterium*	Bacillaceae

on the nematode cell wall) (Cretoiu et al. 2013). A high abundance of this group of bacteria was also observed by Andreo-Jimenez et al. 2021 in *Rhizoctonia* suppressive soil indicating their role in managing biotic stress.

Generic variation between the two treatment sets in both culture-independent and dependent studies

At the genera level, *Herbaspirillum* was the most abundant genus in both groups. Its high abundance in the non-infected group can be reflected by the fact that this genus is reported as a nitrogen fixer that promotes plant growth of rice (Gyaneshwar et al. 2002; James et al. 2002). The association of this genus with infected roots can be attributed to the fact that this bacterium has also been reported as a nematode symbiont that is beneficial to host nematodes (Toju and Tanaka 2019). Although this genus was the most abundant in metagenomic analysis between the two groups, we could not isolate this genus in our culture-dependent analysis. This may be because we have selected the dominant culturable bacterial isolates where other genera have prevailed, or it could be due to an error in our selection procedure.

Some other genera found in our study, like *Chryseobacterium*, *Gemmata*, *Sphingobium*, *Pasteuria*, *Rhizobium*,

and *Pseudomonas*, were specific to infected roots. Their unique presence in gall revealed they might have been associated with the nematode. Reports indicate that these genera have been abundantly found to be associated with *Meloidogyne* sp. or are reported as gall-associated bacteria (Cao et al. 2022; Engelbrecht et al. 2021; Liu et al. 2016). *Chryseobacterium*, *Rhizobium*, *and Pseudomonas* could also be isolated from gall in our culture-dependent studies. Cao et al. 2022 have reported that the abundance of *Chryseobacterium* increased as a result of infection. This genus is also an efficient plant growth promoter (Montero-Calasanz et al. 2014). It may be possible that plants have secreted specific root exudates to recruit this bacterium as a mechanism to promote better growth under nematode infection. The abundance of Pseudomonas in infected gall can also be explained by this phenomenon, as this genus is a vivid plant growth promoter and can also act as a biocontrol agent (Sharifi Noori and Mohd Saud 2012). The genus *Gemmata* also shares an interesting relationship with *Meloidogyne* sp. As the density of the Meloidogyne population increases, the abundance of *Gemmata* decreases (Engelbrecht et al. 2021). This genus is reported to have played a role in promoting cucumber growth (Li et al. 2022). The genera that were unique to non-infected roots were mainly composed of plant growth promoters like *Bacillus*, *Klebsiella*, *Pantoea*, *Delftia*, *Azorhizobium* and *Acidovorax*. Several of these genera are also part of the rice core microbiome and are thus ubiquitously associated with rice (Hussain et al. 2018; Kunda et al. 2018; Shahzad et al. 2017; Zhang et al. 2019). *Acidovorax*, *Azorhizobium*, *Bacillus*, *Delftia*, are well-known nitrogen fixers and act as vivid plant growth promoters (Kunda et al. 2018; Yoneyama et al. 2019). Genera like *Bacillus*, *Klebsiella*, *Delftia* and *Acidovorax* also play a vital role as biocontrol agents against fungal pathogens and nematodes (Lambrese et al. 2018; Li et al. 2017; Liu et al. 2018; Padgham and Sikora 2007; Portieles et al. 2021). Intriguingly, from our culture-dependent studies, all of these unique genera are found to possess maximum indirect plant growth-promoting properties, and they also performed better than the galled isolates in nematode mortality. Hence, it can be concluded that the ecology in the gall-associated microbiome is dominated by bacterial communities that are either associated with nematodes or are enriched with genera that assist plants to withstand stressful environments, whereas the non-infected root microbiome is composed of genera that represent rice microbiome or microbes that have plant growth promoting or biocontrol efficacy. Metagenomic analysis confirmed that different microbial inhabitants occupy different niches in case of infection where the host provides advantage to some while others are hindered. Overall, we made an interesting observation that characteristics of the isolates with regard to their plant growth promoting potential as observed in our metagenomic studies were similar with the culture dependent outcome. The genera unique to the infected roots as identified from the culture-independent techniques had plant growth promoting (PGP) abilities while genera from non-infected roots had strong biocontrol potential in addition to PGP. This pattern was also maintained in our culture dependent work where biocontrol potential was mainly possessed by non-infected isolates and vice versa.

Biological control abilities of bacterial isolates

Over the years, the search for a potential biological control agent against *Meloidogyne* sp. has intensified as many researchers have scrutinised bacteria to test their efficacy under both in vitro and in vivo conditions (Table S5). Although most of these works are focussed on *M. incognita* very few works have been done on *M. graminicola*. It was established that only some strains of *Bacillus* and *Pseudomonas* were capable of exhibiting more than 90% nematicidal activity against *M. incognita* and *M. javanica* (Gao et al. 2016; Hussain et al. 2018; Migunova et al. 2021; Samaliev et al. 2000; Viljoen et al. 2019; Zhao et al. 2018). Among the studies on *M. graminicola*, nematicidal activity against J2 was observed for 60–78% efficacy with strains of *Bacillus* and *Pseudomonas* (Haque et al. 2018; Seenivasan et al. 2012). Screening of all our isolates against *Meloidogyne graminicola* has revealed that the bacterial strains associated with non-infected roots have a higher potential in inhibiting nematodes under laboratory conditions. The genus *Enterobacter* (isolated from SSNI) performed the best among all isolates and showed nematicidal activity of 90%. This is the first report that recorded the efficacy of *Enterobacter* on *M. graminicola*. There are few reports that have documented the role of *Enterobacter* in managing nematode infection (Oh et al. 2018; Zhao et al. 2022). The strain *Enterobacter asburiae* HK169 has been reported as a promising nematicidal agent against *Meloidogyne incognita* by Oh et al. 2018. Regarding the mode of action against nematodes most bacteria have either shown induced systemic resistance in the host or have produced secondary metabolites that have promoted nematode killing (Adam et al. 2014; Anita and Samiyappan 2012; Gao et al. 2016; Oh et al. 2018; Padgham and Sikora 2007). We have screened our isolate by both its ability to produce secondary metabolites that have nematicidal activity as well as by studying if the cells itself are responsible for causing nematode mortality. From our data we can hypothesize that our strain might be producing some metabolites that have been responsible for the nematicidal activity. The *Enterobacter* strain

isolated by us may have several other properties, and to exploit its full potential, detailed studies are required.

Also, the use of *Enterobacter* sp. as a biological control agent is beneficial because not only is it safe to use for the environment, but, at the same time, it can effectively eliminate the target organism. These organisms are non-toxic and can also induce systemic resistance in their host in an effective way. Many species of *Enterobacter* are now being used for successful disease management in greenhouse and field conditions to control many pathogens like, *Rhizoctoia solani*, *Pythium* sp., etc. (Sharma et al. 2017). A few species of *Enterobacter* are also reported to produce the antibiotic Pyrrolnitrin and pseudane against a few specific target pathogens (Sharma et al. 2017). However, there are some cons, which include that the time taken by biological control agents to provide remedies is comparatively longer than chemical pesticides. Another disadvantage is how these organisms might affect the indigenous microbial community structure. We have planned future work to understand the effect of these microbes on the indigenous microflora of a particular soil or region.

Conclusion

i) From our study, we can conclude that infestation by the nematode has drastic effects on plant microbiome and has changed the microbial community composition in the case of gall. Microbial richness was significantly reduced in the case of gall, which was a result of infection.
ii) Genera like *Chryseobacterium*, *Rhizobium*, *Herbaspirillum* and *Pseudomonas* that were prevalent in gall were either associated with nematode and have entered gall as nematode symbiont or may have been recruited by plants as a mechanism to promote its growth under infection. Few genera that are unique to the non-infected root microbiome, like *Bacillus*, *Klebsiella*, *Pantoea*, *Delftia*, *Acidovorax*, and *Azorhizobium*, are reported to have better roles in plant growth promotion as well as in biological control.
iii) From the non-infected root *Enterobacter* sp. strain SSNI 8 acted as a potent biological control agent in inhibiting *M. graminicola* using both its cell culture and cell-free extracts. However, further studies are required to confirm the mode of action of this strain in inhibiting nematodes.
iv) Results obtained from metagenomic studies were validated by culture-dependent techniques. The ecology of non-infected roots is dominated by isolates that can act as potential biological control agent in addition to their plant growth-promoting properties. In contrast, the gall microbiome was dominated by isolates possessing mainly PGP properties.
v) We are planning to do in vivo studies and also have planned to understand how these bacteria interact with plants to prevent nematode infection.

Abbreviations

ACC	1-aminocyclopropane-1-carboxylic acid
ANOSIM	analysis of similarity
BCA	biological control agent
BEPB	beef extract peptone broth
BLAST	basic local alignment search tool
HCN	hydrogen cyanide
IAA	indole acetic acid
invS	inverse simpson index
J2	second stage juvenile
M. graminicola	*Meloidogyne graminicola*
NH3	ammonia
NMDS	non-metric multidimensional scaling
OTU	operational taxonomic unit
PCR	polymerase chain reaction
PGP	plant growth promoters
PPN	plant parasitic nematode
SSI	infected or galled set
SSNI	non-infected or healthy set

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13213-024-01789-0.

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Data availability
The raw sequence metagenome data reported in this paper were submitted to NCBI with Bioproject accession numbers PRJNA478319 and PRJNA478489. The nucleotide sequences obtained with culture-dependent study were also submitted to GenBank with accession numbers OP271491-OP271520.

Authors' contributions
Conceptualization: Pranamita Kunda, Abhishek Mukherjee and Paltu Kumar Dhal; Methodology: Pranamita Kunda, Sandip Mondal and Debjit De; Formal analysis and investigation: Pranamita Kunda and Paltu Kumar Dhal; Writing – original draft preparation: Pranamita Kunda; Writing – review and editing: Paltu Kumar Dhal; Supervision: Paltu Kumar Dhal and Abhishek Mukherjee.

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Data availability
The datasets used or analyzed during the preparation of the manuscript are available from the corresponding author at reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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