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Bacterial community of brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae) revealed by high throughput amplicon sequencing

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Abstract

The bacterial symbionts of brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae), a key sucking insect pest of rice in India and Asia, have been known to play many important physiological functions. Reports of yeast-like symbionts of *N. lugens* are widely known, but little is known about the bacterial microbes. In this study, the bacterial community structure and diversity were examined in *N. lugens* collected from four major rice-growing regions of India utilizing culture-independent high throughput sequencing. The Mi seq technology identified a total of 1277 operational taxonomic units (OTUs) representing 4 phyla mainly (*Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacteroidetes*) by analyzing 16S rDNA gene libraries. The major microbial groups were similar in the four samples, but their distribution patterns were different, especially in Raichur. While the top three bacterial genera linked to Imphal, Pantnagar and Raichur were *Methylobacterium*, *Sphingomonas* and *Acinetobacter*; *Wolbachia* accounted for 87.46 % of the total genera found in Raipur. The identified dominant microbial groups have been known for their crucial role in insect's life cycle. Diversity analysis tests revealed Raichur has the highest species diversity as determined by the high Shannon and Simpson index. According to ACE and Chao1 diversity estimates, Pantnagar has the highest species richness. Understanding the bacterial communities and studying their functional roles will help in formulating biological control strategies specific to this sucking pest.

Keywords

insect microbiome; BPH symbionts; gut microflora; rice planthopper; 16S rDNA

Introduction

Brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae), is a serious sucking insect pest of rice across the world (1, 2). Both BPH nymphs and adults harm rice plants by sucking phloem sap from the sheaths at the base of the plants just above the water level, causing yellowing of the leaves, reduced tiller size, decreased plant height and many empty grains along with "hopper burn" under intense attack (3). Indirect agricultural losses are also caused by viral infections carried by BPH due to grassy stunt, ragged stunt and wilted stunt diseases (4).

In order to overcome survival challenges, insects have evolved integrative strategies through their microbial associations as symbionts in their gut and cells by controlling foraging behaviour, enhancing resistance to pathogens and natural enemies, digesting recalcitrant food sources, provisioning vitamins and metabolising xenobiotic substances (5). Similarly, BPH hosts symbiotic bacteria

and fungi that are essential for its longevity, growth, reproduction, speciation and viability (6). Following an antibiotic pretreatment, BPH became much more sensitive to a variety of pesticides, including imidacloprid, chlorpyrifos and clothianidin. This was due to a reduction in enzyme detoxification process, which was aided by gut microorganisms (7). There is still much to learn about the overall structure of symbionts and their role in the process of BPH adaptation to rice plants (8).

Thus, understanding the functions that bacterial symbionts play in host plant resistance, insecticidal resistance and other processes will be crucial. This can be achieved through the identification and characterization of bacterial symbionts (9). Considering the important roles that symbionts play in BPH, the control of symbionts has been proposed as a potential BPH management strategy (10-12). The diversity and frequency of symbiont infections in insects have been shown to depend on various factors, including host, sex, biotype and geographic area (13). The advancement of metagenomic techniques and their combination with functional multi-omics has made it feasible to functionally represent bacteria in plants and insects, which was difficult to achieve using culture-based methods (14).

The 16S ribosomal RNA gene, or 16S rDNA, is generally employed as a molecular marker for the identification of microorganisms, including those in BPH (15). Due to its conserved nature and the presence of hypervariable regions, the 16S rRNA gene allows accurate identification by comparing species-specific sequences up to strain level. As a result, this gene has emerged as a key marker in microbial ecology and phylogenetic studies of insects (16). Using Next Generation Sequencing (NGS) methods, metagenomic examination of the variable regions of this gene has assisted in the resolution of bacterial populations into operational taxonomic units (OTUs), which were difficult to examine using non-culturable methods (16, 17).

For now, detailed comparative studies of the microbiota associated with BPH utilizing-metagenomic enabled non-culturable techniques are lacking. Keeping in mind that insect microbial associates act as significant resources that may be applied to the biological management of several insect pests (11), the current investigation was conducted to characterize and compare the bacterial symbionts associated with BPH into several taxonomic groups and the effect of geographical area on their bacterial microbiome composition using culture-independent 16S rDNA gene sequencing approach.

Materials and Methods

DNA extraction of BPH samples

BPH samples were collected from four main rice growing areas of India (East, North, Central and South) such as Imphal (IMP), Pantnagar (PNR), Raipur (RAI) and Raichur (RCH), respectively with GPS Coordinates: 24.8170° N, 93.9368° E, 29.0222° N, 79.4908° E, 21.2514° N, 81.6296° E and 16.2160° N, 77.3566° E, respectively. An individual female from each region was used for the analysis of bacterial microbiome during the study year 2021-22. Total DNA was extracted from a single BPH using DNAeasy® Blood and Tissue Kit (Qiagen) in accordance with the manufacturer's instructions with certain modifications in preparation for 16S rDNA gene amplicon sequencing (18). The DNA quantification (A260/A280) ratio obtained for IMP, PNR, RAI and RCH were 1.78, 1.80, 1.94 and 2.0, respectively, which ensured the purity of the DNA samples.

PCR amplification of 16S rDNA gene

The 16S rDNA hypervariable V3-V4 region was amplified using the special barcode primers F (5′- AGAGTTTGATGMTGGCTCAG -3′) and R (5′- TTACCGCGGCMGCSGGCAC -3′). High-fidelity DNA polymerases, 3.2 mM $MgCl_2$, 0.5 mM dNTPs, PCR enzyme buffer and 1 µl template DNA were used to perform PCR along with 40 ng of the isolated DNA and 10 pM of each primer. Thermal cycling was done in 25 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 15 s and elongation at 72°C for 2 min, with the final elongation at 72°C for 10 min. The amplified products were distinguished by electrophoresis on 1.5 % agarose gel. High-quality amplicons from each sample were then pooled in equal amounts according to Biokart India Pvt. Ltd. (Bangalore, India) protocols and submitted for sequencing on the Illumina MiSeq platform with a 2x300PE v3 sequencing kit.

Sequencing protocol

Using barcoded Illumina adapters, 8 cycles of PCR were carried out in order to create the sequencing libraries. The quality of the raw data was evaluated for processing trimmed reads, OTU abundance computation and estimation correction. Quality control (QC) tests were performed on raw sequence data from high throughput sequencing pipelines using FastQC (version 0.11.2) and MultiQC (version 1.9). MultiQC compiled the FastQC findings into a single report. Each read was assigned a category based on identification and percent coverage. Using the UPARSE-OTU pipeline of the 'usearch v11' program, high-quality sequences were classified into OTUs with 97% similarity (19). The Abundance-based Coverage Estimator (ACE) and Chao1 alpha diversity indices were computed to evaluate the richness of microbial communities in BPH samples. To evaluate the diversities, the Fisher's alpha, Shannon and Simpson diversity indices were computed (24).

Results

Overall composition of bacterial communities

A total of 1277 OTUs were recovered after the adaptors, low-quality sequences, overlapping PE reads and chimera were removed. An overview of the microbial community found in the given samples is presented in the OTU table (Table 1).

The results showed that the bacterial communities' diversity of the 4 samples differed significantly. A total of 405 bacterial OTUs were included in IMP, with 0.4 million reads and they were annotated into 17 phyla, 34 classes, 70 orders, 130 families and 194 genera (Table 1). *Methylobacterium*, *Sphingomonas*, *Stenotrophomonas*, *Arsenophonus*, *Acinetobacter*, *Pseudomonas*, *Deinococcus*, *Staphylococcus*, *Bacillus* and *Phenylobacterium* were the top 10 bacterial genera, listed in decreasing order (Fig. 1). Over 99 % of all bacterial phyla were represented by the top 5 main phyla of IMP, which were *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Deinococcus-Thermus* (Table 2).

There were 0.4 million numbers read in PNR with 313 bacterial OTUs in all, broken down into 19 phyla, 40 classes, 82 orders, 149 families and 236 genera (Table 1). The top 10 genera from the sample were *Methylobacterium*, *Sphingomonas*, *Acinetobacter*, *Staphylococcus*, *Pseudomonas*, *Stenotrophomonas*, *Deinococcus*, *Brevundimonas*, *Bacillus* and *Phenylobacterium* (Fig. 1). *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Bacteroidetes* and *Cyanobacteria* were the top 5 main phyla in PNR, accounting for almost 90% of all bacterial phyla (Table2).

A total of 0.2 million reads were recorded in RAI. 248 bacterial OTUs in all, divided into 16 phyla, 31 classes, 63 orders, 117 families and 157 genera (Table 1). RAI showed a substantially different bacterial diversity, with *Wolbachia* (87.46 %) constituting most of the bacterial genera. *Wolbachia*, *Sphingomonas*, *Methylobacterium*, *Acinetobacter*, *Deinococcus*, *Stenotrophomonas*, *Pseudomonas*, *Acetobacter*, *Trueperella* and *Phenylobacterium*ranked among the top 10 genera of the sample in decreasing order (Fig. 1). Over 99 % of the total bacterial phyla identified were represented by the top 5 phyla in RAI, which were *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Deinococcus-Thermus* (Table2).

In RCH, there were 0.2 million reads with 311 bacterial OTUs in all, divided into 17 phyla, 36 classes, 76 orders, 147 families and 234 genera (Table 1). *Methylobacterium*, *Acinetobacter*, *Sphingomonas*, *Bifidobacterium*, *Pseudomonas*, *Corynebacterium*, *Stenotrophomonas*, *Deinococcus*, *Prevotella* and *Bacillus* were the top 10 organisms at the genus level, in decreasing order (Fig. 1). Over 99 % of the total bacterial phyla were represented by the top 5 phyla in RCH, which were *Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Bacteroidetes* and *Deinococcus-Thermus* (Table 2).

Diversity estimates

Overall, for bacterial communities, IMP (405) had considerably more OTUs than other samples (Table 1). The highest species diversity was found in RCH, according to diversity estimates, which were interpreted using high Shannon (3.317) and Simpson indices (0.925). However, according to ACE and Chao1 estimates, PNR has the greatest species richness, with values of 244 and 246.1, respectively. The RAI may have a limited diversity because of the low number of OTUs and species richness/diversity (Table 1).

Comparative analysis of the top 10 bacteria from different taxa

The relative abundance of bacteria (≥1%) at phylum, class, and order levels in each sample is shown. *Proteobacteria* were the most prevalent phylum in all 4 samples, with a maximum relative abundance of 95.31 % in IMP and a minimum of 63.75 % in RCH (Table 2) (Fig. 2 & 3). Its relative abundance in PNR was 67.13 %, but it was 94.85 % in RAI. The relative abundance of *Firmicutes*, the following dominating phylum, ranged from 1.95 % in RAI to 13.14 % in RCH. *Actinobacteria* and *Bacteriodetes*, two other phyla, had comparable relative abundances. *Cyanobacteria* and *Deinococcus-Thermus* were the following two dominating phyla. The remaining phyla had relative abundances of 1% or less in the 4 BPH samples.

The class-level distribution of bacteria identified in four samples is depicted in Table 2 and Fig. 2 & 3. The results showed that except for RAI (3.04 %), the class *Gammaproteobacteria* was found to be the most prevalent in all the samples, accounting for 88.44 %, 30.25 % and 24.49 % in IMP, RCH and PNR, respectively. *Alphaproteobacteria*, with an abundance of 91.16 %, 39.71 %, 30.92 % and 6.24 % in RAI, PNR, RCH and IMP, respectively, were the second most prevalent bacterial class.

The distribution of bacteria present in the four samples at order level is presented in Table 2 and Fig. 2 & 3. According to the results, the order *Enterobacterales* was most common across all samples, particularly IMP, accounting for 86.82 % abundance, along with 13.30 % and 10.39 % in RCH and PNR, respectively, with RAI (1.18 %) being the exception. *Rickettsiales* was the second most prevalent bacterial order in RAI, with 83.94 % abundance. *Rhizobiales*, which contributed 17.39 %, 13.83 %, 3.01 % and 2.91 % of the total bacterial population in PNR, RCH, RAI and IMP, respectively, was the third dominating order.

Results at the family level showed that *Morganellaceae* accounted for 87.36 % in IMP but less than 2 % in the other samples (Table 2). *Anaplasmataceae*, in contrast to other samples where it was less than 1 %, was the dominant family in RAI with 85.32 % relative abundance. *Sphingomonadaceae* and *Methylobacteriaceae* both have equivalent relative abundances in the samples.

The bacterial community compositions of all four samples, except for RAI, were relatively similar at the genus level (Table 2) (Fig. 2). The top three bacterial genera linked to IMP, PNR and RCH were *Methylobacterium*, *Sphingomonas* and *Acinetobacter*. *Wolbachia* accounted for 87.46 % of the total genera found in RAI, yet this only accounts for less than 1 % of the total bacterial diversity across all other samples. The next major genus was *Methylobacterium*, which was 23.37 % of the total bacterial populations in IMP, 21.09 % in PNR, 15.09 % in RCH and 2.77 % in RAI. In decreasing order, the further bacterial genera that were extensively associated with 4 BPH samples (relative abundance >1 %) were *Sphingomonas*, *Pseudomonas*, *Stenotrophomonas*, *Staphylococcus*, *Deinococcus*, *Bifidobacterium*, *Bacillus*, *Corynebacterium*, *Phenylobacterium*, *Acetobacter*, *Paracoccus*, *Lactobacillus*, *Brevundimonas* and *Serratia*.

Table 1. Numbers of different taxonomic categories and alpha diversity indices of bacterial communities obtained from 4 Brown planthopper samples

Sample ID	Number of reads	Number of OTUs	Number of different taxonomic categories					Alpha diversity Indices				
			Phylum	Class	Order	Family	Genus	Simpson	Shanon†	Fisher's alpha	Chao 1	ACE
IMP	0.4M	405	17	34	70	130	194	0.8953	3.198	31.49	204.1	207.1
PNR	0.4M	313	19	40	82	149	236	0.9104	3.309	29.33	246.1	244.0
RAI	0.2M	248	16	31	63	117	157	0.2332	0.7646	19.06	167.1	167.1
RCH	0.2M	311	17	36	76	147	234	0.925	3.317	30.06	238.6	238.3

Table 2. Relative abundance of top 10 bacteria at phylum, class, order, family and genus level in different Brown planthopper samples

Phylum

Sample ID	Proteobacteria	Firmicutes	Actinobacteria	Bacteroidetes	Deinococcus-Thermus	Cyanobacteria	Chloroflexi	Fusobacteria	Planctomycetes	Gemmatimonadetes
IMP	95.31	2.12	0.77	1.23	0.39	0.02	0.04	0.03	0.01	0.03
PNR	67.13	13.07	7.95	5.58	1.86	3.47	0.25	0.15	0.12	0.13
RAI	94.85	1.95	0.85	1.41	0.66	0.18	0.02	0.01	0.04	0.00
RCH	63.75	13.14	14.25	6.35	1.93	0.14	0.16	0.06	0.04	0.02

Class

	Gammaproteobacteria	Alphaproteobacteria	Bacilli	Actinobacteria	Betaproteobacteria	Clostridia	Bacteroidia	Chitinophagia	Deinococci	Flavobacteriia
IMP	88.44	6.24	1.61	0.73	0.45	0.47	0.46	0.39	0.39	0.19
PNR	24.49	39.71	10.35	8.13	4.57	2.88	1.83	2.53	1.92	0.68
RAI	3.04	91.16	1.43	0.84	0.63	0.49	0.53	0.62	0.66	0.11
RCH	30.25	30.92	10.09	14.19	2.06	2.86	3.21	1.69	1.94	0.93

Order

	Enterobacterales	Rickettsiales	Rhizobiales	Sphingomonadales	Pseudomonadales	Bacillales	Lactobacillales	Micrococcales	Caulobacterales	Clostridiales
IMP	86.82	0.04	2.91	2.44	0.93	0.95	0.66	0.35	0.43	0.47
PNR	10.39	0.03	17.39	13.56	10.08	6.26	3.74	4.45	3.87	2.78
RAI	1.18	83.94	3.01	3.10	1.22	0.71	0.72	0.27	0.42	0.48
RCH	13.30	0.02	13.83	11.50	13.51	5.59	4.49	3.00	2.57	2.86

Family

	Morganellaceae	Anaplasmataceae	Methylobacteriaceae	Sphingomonadaceae	Moraxellaceae	Enterobacteriaceae	Caulobacteraceae	Pseudomonadaceae	Staphylococcaceae	Xanthomonadaceae
IMP	87.36	0.04	2.70	2.49	0.54	0.27	0.44	0.41	0.32	0.60
PNR	0.75	0.03	17.51	15.33	7.94	4.49	4.39	3.48	3.82	2.67
RAI	0.05	85.32	2.76	3.15	0.84	0.53	0.43	0.39	0.28	0.54
RCH	1.74	0.01	13.40	13.15	11.91	5.28	2.97	3.68	2.13	2.78

Genus

	Wolbachia	Methylobacterium	Sphingomonas	Acinetobacter	Pseudomonas	Staphylococcus	Stenotrophomonas	Bifidobacterium	Deinococcus	Corynebacterium
IMP	0.35	23.37	19.35	4.32	3.53	2.66	5.05	0.14	3.46	1.03
PNR	0.04	21.09	16.58	9.04	4.28	4.48	3.06	0.20	2.64	1.40
RAI	87.46	2.77	2.82	0.78	0.39	0.26	0.52	0.03	0.69	0.11
RCH	0.02	15.09	13.39	13.80	4.11	2.37	2.98	8.01	2.70	3.85

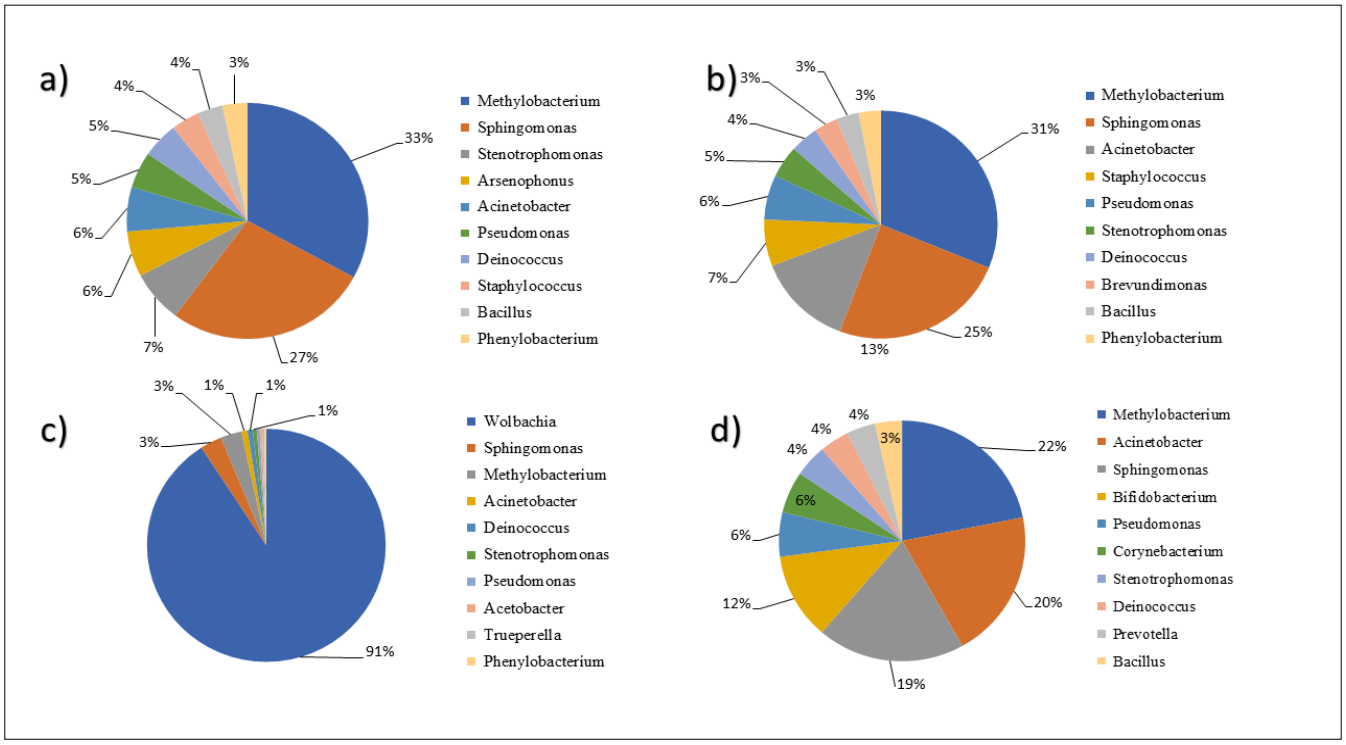

Fig. 1. Top 10 genera of bacterial symbionts in different samples of Brown planthopper. a) Imphal, b) Pantnagar, c) Raipur and d) Raichur

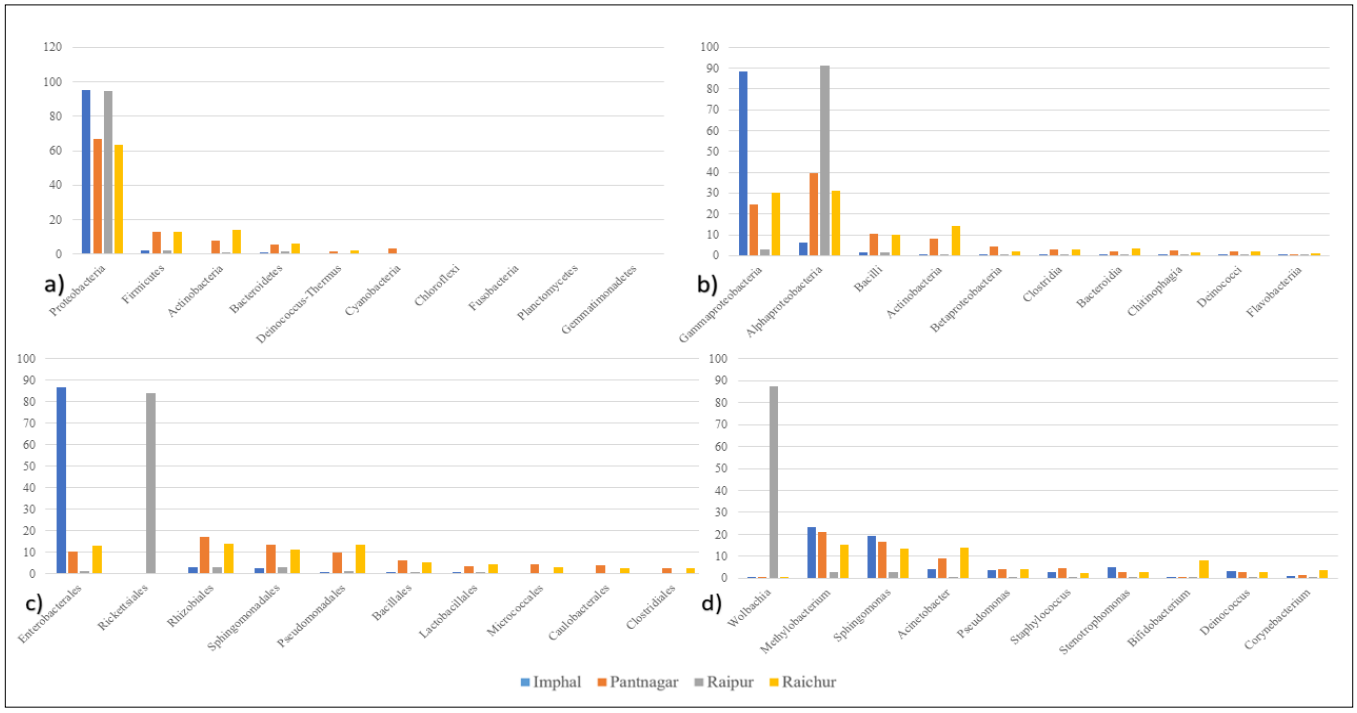

Fig. 2. Relative abundance of top 10 bacteria at a) phylum, b) class, c) order and d) genus level in 4 Brown planthopper samples

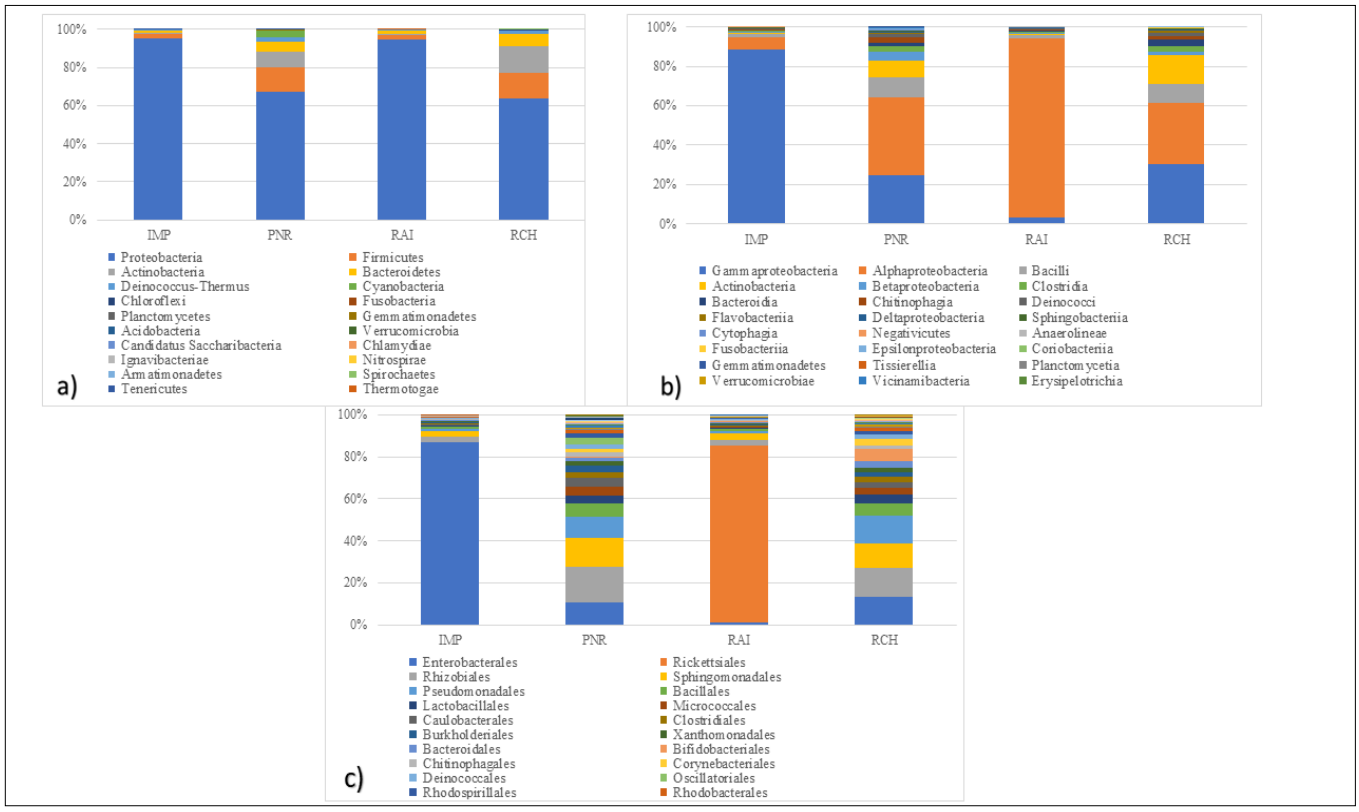

Fig. 3. Relative abundances of all identified bacteria at a) phylum, b) class and c) order level in 4 Brown planthopper samples; IMP=Imphal, PNR=Pantnagar, RAI=Raipur and RCH=Raichur

Bacterial diversity based on Venn diagram and heat map

The findings of the Venn diagram representing the unique and shared bacterial genera for four BPH samples revealed that 315 genera were obtained from all four samples (Fig. 4). In all, 194, 236, 157 and 234 different bacterial genera were found in the IMP, PNR, RAI and RCH samples, respectively. From the matching samples, the unique genera were 23, 36, 4 and 34, respectively. Among these, 110 bacterial genera, which were 34.92 % of the respective genus repertoires, were common to all samples.

The patterns of microbial communities vary significantly at the genus level throughout the 4 samples, as shown by the heat map of the 20 most abundant bacteria (Fig. 5). Furthermore, the abundances of the most prevalent taxa varied considerably among samples. Particularly, PNR had considerably greater relative abundances of *Brevundimonas*, *Sphingomonas*, *Methylobacterium*, *Phenylobacterium*, *Serratia*, *Staphylococcus* and *Streptococcus* than other samples. Similar to this, RCH had much greater relative abundances of *Bifidobacterium*, *Corynebacterium* and *Prevotella* than others. *Wolbachia* had the most strikingly divergent pattern of relative abundance, with high relative abundance in RAI (87.46 %) and insignificant in others (<1 %).

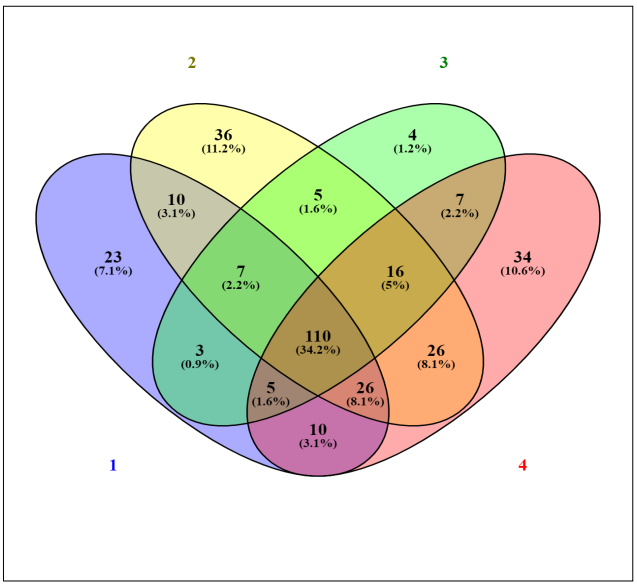

Fig. 4. Venn diagram depicting common and unique bacterial genera in 4 samples of Brown planthopper; 1) Imphal, 2) Pantnagar, 3) Raipur and 4) Raichur

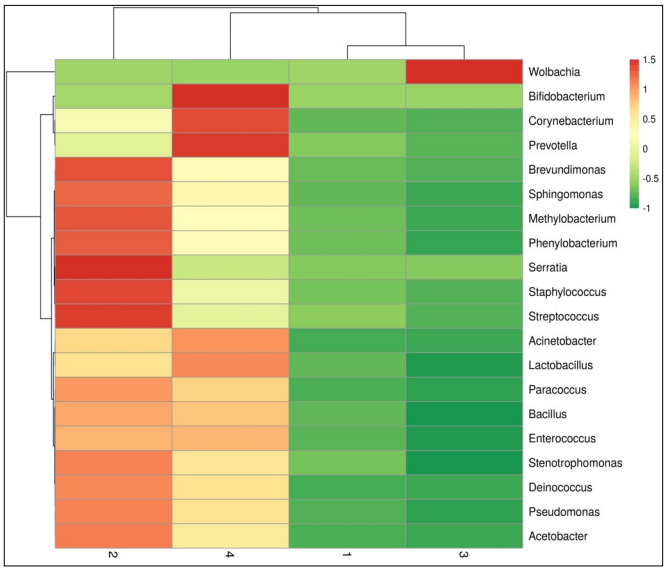

Fig. 5. Heat map showing bacterial abundance of top 20 dominant genera in 4 samples of Brown planthopper; 1) Imphal, 2) Pantnagar, 3) Raipur and 4) Raichur

Discussion

Characterization and evaluation of the bacterial community structures and diversity in BPH were done using Illumina MiSeq high-throughput sequencing of the bacterial 16S rDNA gene. Previous studies used culture-dependent methodologies together with conventional molecular approaches to study the microbial symbionts of BPH (17, 20). Recent research has provided a clearer understanding of the microbial communities linked to BPH via high-throughput sequencing techniques (21, 22).

Bacterial diversity estimation

More than 90 % of the microflora in the environment cannot be cultivated and is, as a result, unexplored. In this work, the 16S rDNA gene was sequenced using Illumina MiSeq, a culture-independent approach that gives precise data on bacterial populations associated with insects (23, 24).

Our investigation showed that the BPH microbiome was diverse and abundant. Based on species similarity of >97 %, 1277 OTUs were uncovered. The level of taxonomical classification for bacteria was significantly higher than what had been seen in other studies using conventional molecular methods (20, 25). Consequently, 110 common genera were found in all 4 samples, which represented various agroecological zones, out of a total of 315 genera. High-throughput sequencing technology was previously used to characterize bacterial populations in diverse BPH tissues (26).

According to the diversity estimates, RCH (Table 1) revealed the highest species diversity, as determined by high Shannon and Simpson index. According to ACE and Chao1 diversity estimates, PNR had the highest species richness. This indicates that the sample may have limited diversity, as the RAI had a remarkably low Simpson diversity score (0.23). Group-wise variations in the microbiome of *Hermetiai llucens* based on their sampling day and environment were also observed (27, 28). Due to its restricted diet, which prevents BPH from obtaining nutrients on its own, it must rely on symbionts, which results in a high bacterial diversity (6).

Molecular characterization of the bacterial symbionts

Based on the 16S rDNA study, the majority of BPH bacteria were *Proteobacteria*, ranging from 63.70 % in RCH to 95.30 % in IMP. *Proteobacteria* was shown to be the most common phylum during all phases of BPH development (23, 29). The next three prominent phyla in all the samples were *Firmicutes*, *Actinobacteria* and *Bacteriodetes*. Likewise, *Firmicutes*, *Actinobacteria* and *Bacteroidetes* were also abundant in *N. lugens*, *Saperdavestita* and *Reticulitermes speratus* (30, 31). *Firmicutes* dominated the ovary and fat body of BPH (26).

With the exception of RAI, *Methylobacterium*, *Sphingomonas* and *Acinetobacter* were found to be the top three bacterial genera associated with BPH samples. *Wolbachia* was detected in RAI at a relative abundance of 87.46 %. *Wolbachia* was the most prevalent bacterial genus in *Sogatella furcifera* and *Laodelphax striatellus* (32). *Wolbachia* and *Arsenophonus* were mostly present in fat bodies and were passed through maternal lines in *N. Lugens* (33). *Wolbachia* is widely known for playing important roles in cytoplasmic incompatibility, which inhibits BPH proliferation (10). The similarity of the microbiota in BPH samples from different geographical areas shows that some bacteria are more crucial to its growth and development and these specific bacterial genera are important for preserving host health (34). *Arsenophonus* dominated the bacterial populations in BPH, while *Wolbachia* was found in very low concentrations (35). Intriguingly, our study did not find *Arsenophonus* to be a dominant bacterium; instead, it made up just 4.41 % of all the bacteria in IMP and was scarce in other samples (relative abundance <0.1 %).

The bacterial composition of BPH was found to be significantly different for RAI than for IMP, PNR and RCH. Although RAI had less bacterial variety and abundance than other samples, the precise cause is yet unclear. Similar to past studies, the findings showed that varied settings influence the host's microbiota differently, albeit most obligatory symbionts were the same (36, 37). Food supplies and the natural environment change because of geographic shifts. Diverse host diets may cause a change in the microbial structure, which will affect how those organisms function and use their metabolic pathways (38).

It was demonstrated that the bacterial abundance and structure corresponded in populations that were geographically closer together by classifying *L. striatellus* populations into three groups based on their geographic locations, namely temperate, subtropical and tropical (23). Although bacterial and fungal diversity and abundance levels in rice planthopper populations from different regions were rather consistent, their community architectures in *L. Striatellus* and *S. furcifera* varied greatly from region to region (32). The diversity of gut symbiotic bacteria of *Solenopsis invicta* is significantly positively correlated with the geographical distance of the colony. The composition and abundance of host-microbial composition are influenced by both the environment and host genetics and these causes vary geographically (39).

Conclusion

In-depth knowledge about the bacterial communities associated with BPH was highlighted using 16S rDNA gene sequencing of bacterial isolates using Illumina MiSeq. RCH had the highest species diversity, followed by PNR, IMP and RAI. Although diverse environments result in distinct microbiota for BPH, the bulk of the bacteria, which are crucial for the insect's life cycle, were consistent across all samples. Understanding the microbiota that governs BPH-rice interactions may aid in the development of novel, efficient and environmentally friendly methods to minimize its damage to rice crops.

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Authors' contributions

ST carried out the molecular genetic studies, participated in the data analysis, and drafted the manuscript. VA carried out DNA extraction and data curation. SN participated in the study design and performed the statistical analysis. SN, RNS conceived the study, supervised it and participated in its design and coordination. RNS finalized the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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