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Original article

Gut microbial composition in developmental stages of gall inducing thrips *Gynaikothrips uzeli* and associated plant pathogenesis

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ABSTRACT

Gut bacteria play a crucial role in the several metabolic activity of the insects. In the present work, effort has been made to decipher the gut microbiota associated with the developmental stages of *Gynaikothrips uzeli* a gall inducing thrips along with their predicted functional role. Further, an effort has been made to correlate the bacterial communities with plant pathogenesis and thelytoky behaviour of *G. uzeli*. Findings obtained revealed that genus *Arsenophonus* dominated the total bacterial diversity and was transmitted vertically through the developmental stages. Further, it was observed that the high abundance of genus *Arsenophonus* promotes the thelytoky behaviour in *G. uzeli* and results in the killing of males. Furthermore, strong connecting link between *Arsenophonus* abundance and gall induction in *F. benjamina* was observed in the current dataset. *G. uzeli* being in the category of phloem sucking insect was known for the induction of galls and the current findings for the first time unveiled the facts that high abundance of genus *Arsenophonus* a well-known plant pathogen may be one of the major reason for inducing galls in *F. benjamina*. Moreover, PICRUSt2 analysis revealed that predicted functional pathways like biosynthesis of amino acids, and metabolism of carbon, nitrogen, carbohydrates and amino acids (e.g. Arginine, Alanine, Aspartate, Glutamate, Proline, Cysteine, Methionine, Glycine, Threonine, and Serine) were frequently noticed in profiles associated with all the developmental stages of *G. uzeli*. More to this, the high abundance of *Arsenophonus* in *G. uzeli* suggest that representatives of this genus may be resistant and/or tolerant to different antibacterial agents, alkaloids, flavonoids, and glycosides (e.g. quercetin). The correlation of bacterial diversity in pathogenicity can be extrapolated in different pest and vector species of other arthropods.

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1. Introduction

Insects are the most diverse group of organisms, contributing about 66% to the total animal diversity. Due to their complex body structure and feeding habitats, they are present everywhere in the habitats. The insect cells, gut, exoskeleton, and hemocoel are the colonial hub for the proliferation of microorganisms. They play various essential roles such as nutrition, defensive mechanism against pathogens, and metabolizing harmful toxins (Dillon and Dillon, 2004; Douglas, 2015; Koch and Schmid-Hempel, 2011; Shin et al., 2011). These microorganisms are transmitted via two modes i.e. horizontal (transmitted from an environment or free-living symbiont source) and vertical (from mother to offspring) (Bright and Bulgheresi 2010). The symbiotic bacteria such as *Wolbachia, Cardinium*, *Rickettsia*, *Arsenophonus*, and *Spiroplasma* are generally known as "Son-killers" and lead to feminization, parthenogenesis, cytoplasmic incompatibility in many arthropods (Rey et al., 2013).

The wide array of bacterial roles in an insect develop the interest of researchers across the globe to unfold the microbiome research and their functional role in insect's life cycle or any associated pathogenesis. Although insects are one of the most diverse groups of invertebrates, the analyses of microbiota associated with insect species are predominantly performed for economically or

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medically significant species (e.g. pests or disease vectors) (Chen et al., 2016; Gao et al., 2020; Gabrieli et al., 2021). Nevertheless, microbiota profiling has been also performed for selected representatives of butterflies (Minard et al., 2019; Phalnikar et al., 2019), termites (Köhler et al., 2012; Su et al., 2016), ants (Funaro et al., 2011; Poulsen and Sapountzis 2012), firebugs (Sudakaran et al., 2012; Salem et al., 2013), fruit flies (Chandler et al., 2011; Wong et al., 2011), beetles (Reid et al., 2011; Hulcr et al., 2012), bees (Martinson et al., 2011; Engel et al., 2012) etc. Further, such analyses have been also conducted for few species of Thysanoptera such as *Frankliniella fusca* (Wells et al., 2009), *Frankliniella tritici* (Powell et al., 2015), *Hoplothrips carpathicus* (Kaczmarczyk et al., 2018), *Scirtothrips dorsalis* (Dickey et al., 2014), and *Thrips tabaci* (de Vries, et al., 2008; Gawande et al., 2019). Kaczmarczyk et al. in 2018 elucidated the bacterial diversity in the developmental stages of *Hoplothrips carpathicus,* a fungivorous thrips and revealed the presence of 21 bacterial phyla such as *Proteobacteria, Actinobacteria*, *Bacterioidetes, Firmicutes*, *etc.* which was further down the clade classified in taxonomic classes like *Alphaproteobacteria*, *Actinobacteria*, *Gammaproteobacteria*, and *Betaproteobacteria*. Furthermore, the genus *Wolbachia* was found predominating in pupa and adult, contributing ~69.95% and 56.11% of the total bacterial population (Kaczmarczyk et al., 2018).

In the present work, we have investigated the gut microbiome in the developmental stages of *Gynaikothrips uzeli,* a weeping fig thrips that is known for inducing leaf galls in *Ficus benjamina* (ThripsWiki, 2021). Further, *Gynaikothrips* had achieved the pest status as they are damaging the young leaves of ornamental plants like *F. benjamina* and causing severe economic losses to the global horticultural industries (Held et al., 2005; Cambero-Campos et al., 2010; Cavalleri et al., 2011; De Borbo'n et al., 2011; Hoddle and Mound, 2011; Tree 2012; Yu et al., 2012). The young leaves of this ornamental plant after thrips infestation turned yellow with red purplish spots and leaf curls (Held et al., 2005; Cao et al., 2017; Nagrare and Naikwadi 2016). Further, severe infestations cause defoliation and diminutive growth of the host plant and hence reduce the ornamental value of *F. benjamina* (Dara and Hodel 2015). Apart from the ornamental values, *F. benjamina* is known for its medicinal value due to presence of antimicrobial, antinociceptive, antipyretic, hypotensive, and anti-dysentery properties (Imran et al., 2014).

Keeping in view, the gall-inducing behaviour of *G. uzeli* and the high ornamental value of *F. benjamina*, we have investigated the gut bacterial diversity in all developmental stages i.e. larva I, larva II, pupa and adult of- *G. uzeli* along with its role in gall-inducing behaviour. More specifically, the present work will provide answers to the following questions: 1) Gut bacterial diversity in all developmental stages along with their transmission; 2) Predicted functional role of bacterial diversity with respect to their host; 3) bacterial taxa responsible for the plant pathogenesis or gall-inducing behaviour in *G. uzeli*.

2. Material and methods

2.1. Sampling, and identification

The leaf galls of *F. benjamina* were collected from Kolkata, West Bengal state of India. The developmental stages like adults and two feeding stages (i.e. larva I and larva II) were collected from these leaf galls, preserved in 100% ethyl alcohol and stored at 4 °C. Further, the few larval stages were reared in the laboratory for the development of the non-feeding semiquesent pupal stage (Pupa). Specimens of each developmental stage were first rinsed with phosphate-buffered saline (PBS) in order to remove the environmental contamination which later on followed by the lysis step. After the lysis step, the voucher specimens were mounted onto the glass slides for morphological identification by the taxonomic expert in the field of Thysanoptera, Dr. Kaomud Tyagi using published keys (Ananthakrishnan and Muraleedharan, 1974; Ananthakrishnan and Sen 1980).

2.2. DNA Isolation, amplification and sequencing

The obtained specimens (50 each) of adult, larva I, larva II and pupa were subjected to the DNA extraction using DNeasy Blood & Tissue Kit (Qiagen) (cat. nos. 69504) with a manufacturer's protocol. The DNA from each specimen of different developmental stages was isolated and pooled equally into triplicate. Further, the quality and quantity of the DNA were checked using gel electrophoresis and Qubit 2.0 Fluorometer (Q32866, Thermofisher) respectively. For amplification of the extracted DNA, primer sets 341F (5′- ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGAC TACHVGGGTWTCTAAT-3′) of the V3-V4 region were used. The amplification steps involved the reaction mixture 25 µl containing 1 µl each forward-reverse primer, Takara, Taq DNA polymerase (0.5 µl), dNTPs (1 µl), 10x buffer (2.5 µl), template (50 ng), and Milli Q water. The reaction cycle includes denaturation (5 min at 98 °C followed by 35 cycles for 30 s at 98 °C), annealing (45 s at 53 °C), elongation (72 °C for 45 s), and final extension (7 min at 72 °C). Furthermore, the PCR products (n = 12) in triplicate for each developmental stage were sent for sequencing on the Illumina MiSeq platform with 150x2 chemistry. The generated raw reads were submitted under the BioProject ID PRJNA741334 (Accession Number: SAMN19866870- SAMN19866879) to the National Center for Biotechnology Information (NCBI) GenBank Portal.

2.3. Bioinformatics and statistical analyses

The sequenced reads were subjected to the bioinformatics analysis using QIIME2 (ver. 2020.2) (Bolyen et al., 2019). The generated forward and reverse raw reads were merged into a single demultiplexed file. The demultiplexed file was quality trimmed, de-noised, and merged in DADA2 pipeline (Callahan et al., 2016) which was later followed by the chimera removal. Further, the non-chimeric reads were assigned into Amplicon Sequence Variants (ASVs). The DADA2 modified sequence files were used for the taxonomic classification in SILVA database (version 132) with 99% similarity. Upon taxonomic classification, the ASVs file along with taxonomic file and metadata were uploaded on the online web server MicrobiomeAnalyst (Dhariwal et al., 2017; Chong et al., 2020) for downstream analysis. A total of 268 ASVs (200 singletons and 68 with more than two counts) were obtained from the downstream analysis, which was shown in the manuscript with the help of Venn diagram using online tool jvenn (http://jvenn.toulouse.inra.fr) (Bardou et al., 2014). Further, the alpha diversity considering diversity measures such as Chao1, Observed, Shannon, and Simpson with ANOVA statistical method was used to decipher the richness and evenness in bacterial diversity of developmental stages. On the other hand, beta diversity considering Bray Curtis (PCoA) and unweighted UniFrac distance with Ward's linkage (dendrogram) using PERMANOVA based statistical method was used to decipher the distribution and phylogenetic arrangement (based on bacterial diversity) of developmental stages. Moreover, the functional metabolic pathways were predicted using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) (Langille 2018; Langille et al., 2013; Douglas et al., 2020) using Kyoto Encyclopaedia of Genes and Genomes (KEGG) database (Kanehisa and Goto 2000; Kanehisa 2019; Kanehisa et al., 2019). The generated file with metadata was uploaded in the MicrobiomeAnalyst for visualization of predicted pathways (Dhariwal et al., 2017; Chong et al., 2020).

3. Results

3.1. Bacterial diversity through the developmental stages of G. uzeli

To decipher the gut microbiome and their transmission through the developmental stages i.e. larva to adult in *G. uzeli*, we had generated 16S amplicon data sequences. The generated sequences in triplicate ranges possess the minimum of 1, 67,534 raw reads to 2, 34,035 maximum raw reads. The obtained raw reads were quality trimmed, filtered and de-noised in DADA2 pipeline to generate good quality reads (Q value = 20). The rarefaction curve for each developmental stage was saturated, which implies that sufficient sequencing depth was achieved for the further analysis. Obtained reads were classified using SILVA database which resulted in identification of 268 ASVs. The Venn diagram of these 268 ASVs revealed that only 5 ASVs were shared between all the developmental stages, while a total 14 ASVs were unique in adult, 101 in larva I, 96 in larva II, and 15 in Pupa I (Fig. 1a). Larva I possess maximum number (144) of ASVs while adult possess minimum number (23) of ASVs. In terms of sharing of ASVs maximum of 30 ASVs was shared between both the larval stages while minimum number of one ASV was shared between larval and pupa stage.

Further analyses were performed after singletons removal and filtering out the ASVs characterized by low variance and low abundance. In total, 201 ASVs were filtered out from the dataset and 67 ASVs were used for analyses of microbiota transmission between selected developmental stages. Those 67 ASVs were represented by the 42 genera belonging to the 8 phyla. Representatives of the genus *Arsenophonus* belonging to phylum Proteobacteria contributed around 98–99% to the total bacterial communities tested what is in line with the hypothesis of its vertical transmission through all the developmental stages of *G. uzeli* (Fig. 1c). The rest bacterial taxa contributing 1–2% of the total bacterial diversity were represented by the genera such as *Acinetobacter*, *Staphylococcus*, *Pseudomonas*, *Paracoccus*, *Coryneobacterium_1*, *Cutibacterium*, *Enterobacter*, *Wolbachia*, etc. (Fig. 1b).

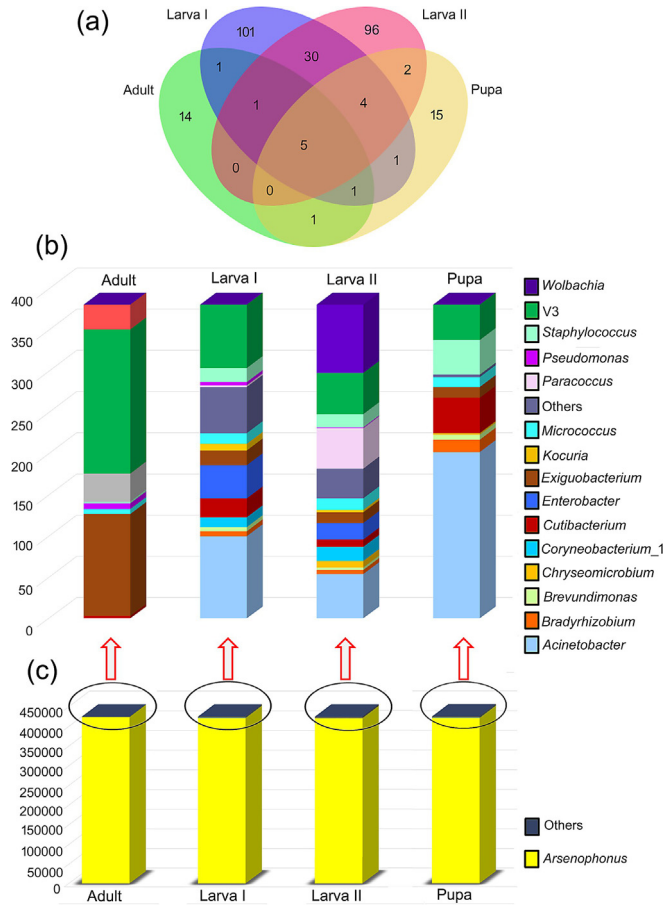

Fig. 1. (a) Venn diagram (b) Percentage contribution of gut microbiota (1–2%) at genera level (After *Arsenophonus* removal) (c) Percentage contribution of gut microbiota at genera level towards total bacterial diversity (with *Arsenophonus*).

3.2. Diversity indices Alpha- and Beta-Diversity

The α-diversity analysis based on four diversity measures (Chao1, Observed, Shannon, and Simpson) was carried out on raw reads to investigate the richness and evenness in the bacterial diversity through the developmental stages of *G. uzeli*. The Chao1 and Observed diversity measure were ranged from 9 to 38, while the values of Shannon and Simpson indices lies in the range of 0.1 to 0.10, and 0.002 to 0.026, respectively (Fig. 2a). The richness (Chao1 & Observed) and evenness (Shannon & Simpson) estimators for all the life stages were non-significant (p-value > 0.05). The α-diversity analysis was also carried out after the removing the *Arsenophonus* reads from the dataset. Obtained results revealed that diversity measure like Chao1 and observed ranged from 5 to 37, while the Shannon and Simpson indices ranged from 1.19 to 2.7, and 0.64 to 0.92, respectively. Furthermore, all the diversity

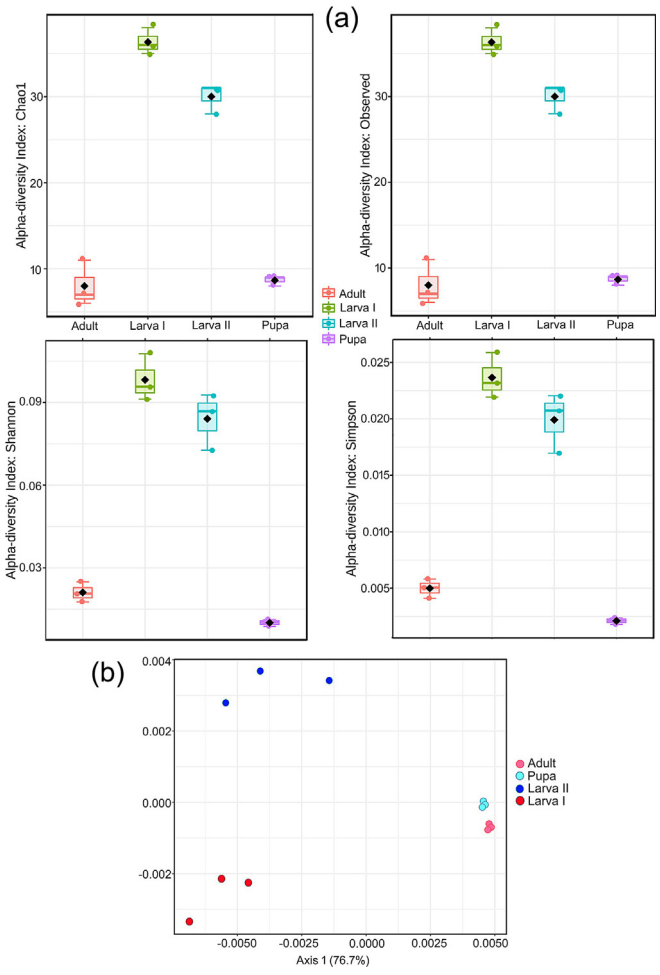

Fig. 2. (a) Box plot for the alpha-diversity index showing observed, Chao1, Shannon and Simpson diversity indices in all the developmental stages of the *G. uzeli*. (b) Bray-Curtis dissimilarity-based PCoA plot deciphering the distribution of bacterial diversity in the all the replicates of the developmental stages in *G. uzeli*.

measures after removing the *Arsenophonus* reads were still non-significant (p-value > 0.05).

Principal Coordinates Analysis based on Bray Curtis distance methods was used to decipher the β-diversity. The replicates of pupa and adult lies closer to each other in comparison to the larva-I and larva-II replicates (p-value < 0.01) (Fig. 2b). Similar results were yielded from the Unweighted UniFrac based dendrogram analysis where adult and pupa lied in the same clade i.e. Clade 1, while larva-I and larva-II lied in Clade-II. Dendrogram analysis indicated that the bacterial diversity of adults resembles closer the bacterial diversity of pupa rather than larval stages.

After removal of *Arsenophonus* reads from datasets for all tested developmental stages, PCoA analysis revealed that the bacterial communities of larva-I, larva-II and pupa are more similar to each other than to profiles determined for adults (p-value < 0.01). This result was also supported by the dendrogram topology revealing two clades: one containing microbiota profiles of adults and second clade containing profiles of two larval stages and pupa. Those results showed that, after removal *Arsenophonus* reads, the bacterial communities associated with *G. uzeli* adults are different from those communities associated with larvae and pupa.

3.3. LEfSe discriminant analysis

Linear Discriminant Analysis (LDA) Effect Size (LEfSe) was implemented to estimate the size effect of each differentially abundant feature through the developmental stages. A total of 16 significant (p-value < 0.01) features were identified. The most differentially abundant bacterial taxa were *Arsenophonus* (LDA = 4.72, P = 0.023), *Exiguobacterium* (LDA = 3.61, P = 0.02), *Staphylococcus* (LDA = 3.42, P = 0.032), and *Micrococcus* (LDA = 3.3, P = 0.029) detected in all the developmental stages. Further, *Acinetobacter* (LDA = 4.22, P = 0.014) and *Cutibacterium* (LDA = 3.47, P = 0.028) were differentially abundant in larva-I, larva-II and pupa, while *Paracoccus* (LDA = 3.76, P = 0.023), *Enterobacter* (LDA = 3.72, P = 0.013), *Staphylococcus* (LDA = 3.42, P = 0.032), *Kocuria* (LDA = 3.13, P = 0.025), *Deinococcus* (LDA = 2.91, P = 0.025), and *Brevibacterium* (LDA = 2.88, P = 0.025) were observed in larva-I, larva-II. In turn, *Lawsonella* (LDA = 2.41, P = 0.013) was differentially abundant only in larva-I, while *Wolbachia* (LDA = 4.05, P = 0.013) and *Weeksella* (LDA = 2.97, P = 0.01) in larva-II; and adult respectively (Fig. 3). Moreover, this analysis indicated that the genus *Arsenophonus* was detected and differentially abundant in all tested developmental stages.

3.4. Predicted functional analysis

The PICRUSt2 predicted the microbiota functions from the 16S rRNA data. Results of this analysis showed that pathways involved in biosynthesis of amino acids, and metabolism of carbon, nitrogen, carbohydrates and amino acids (e.g. Arginine, Alanine, Aspartate, Glutamate, Proline, Cysteine, Methionine, Glycine, Threonine, and Serine) were frequently noticed in profiles associated with all the developmental stages of *G. uzeli*. Moreover, also pathways involved in degradation of amino acids (e.g. Valine, Leucine, and Isoleucine) were identified in all tested microbiota profiles. Among pathways identified in all tested profiles, also those characteristic for particular developmental stage were noticed. For example, the predicted pathway of Galactose metabolism was present only in larva-I, while carbon fixation pathway was present only in larva-II. Predicted functional pathways like phenylalanine metabolism, Benzoate degradation, Fructose and mannose metabolism, amino sugar and nucleotide sugar metabolism were present in larval and pupal developmental stages only (Fig. 4).

3.5. Arsenophonus as son's killer in G. uzeli (symbiotic association)

The leaf galls of *F. benjamina* were keenly observed under the microscope and we did not found a single specimens of the male adult. Males were not observed even after rearing of larval stages. These findings may lead to the conclusion that abundant representatives of genus *Arsenophonus* act as son's killer in *G. uzeli* just like in hymenopterans like *Nasonia* species (Wilkes et al., 2011). Further, it is for the first time in Thysanoptera where microbial genus *Arsenophonus* was reported as son's killer and it will open up new scopes for the researchers to unveil the association of this bacterial taxa with other Thysanoptera species to explore the son killing mechanism.

3.6. Arsenophonus as plant pathogen to F. benjamina

Arsenophonus sp., as known plant pathogen was observed in all the developmental stages of *G. uzeli*. This genus contributed around 98–99% of all identified profiles associated with selected developmental stages of *G. uzeli* that supports the hypothesis of its vertical transmission. Moreover, this result lead to the hypothesis that the gall inducing behaviour of *G. uzeli* in *F. benjamina* may be due to the high abundance of *Arsenophonus*. Further, no concrete evidence is available in the literature that unveils this gall inducing behaviour of *G. uzeli*. Here, for the first time we are revealing that the genus *Arsenophonus* may be responsible for the induction of galls in *F. benjamina*. The proposed mechanism of the leaf galls induction and role of *Arsenophonus* was shown in Fig. 5.

3.7. Discussion

Bacterial communities play a significant role in host interactions, most of the time it is beneficial for the host and sometimes it is harmful i.e. causes sexual altercations and pathogenesis (Koch and Schmid-Hempel, 2011; Engel and Moran 2013). Although *G. uzeli* is an important gall inducing in *F. benjamina,* the information about the interaction of microbiota with this pest and its role in functional metabolism is still missing in the literature. Furthermore, there is no concrete evidence regarding the correlation of bacterial diversity with this gall inducing behaviour of *G. uzeli* in the literature. With the recent advancement in microbiome analysis through NGS, one can find the host-microbe interaction with great ease (Gawande et al., 2019). In the present work, the first detailed investigation has been made to elucidate the bacterial diversity among the developmental stages of the *G. uzeli* using 16S rRNA amplicon sequencing. In addition to this, an effort has been made to link the bacterial diversity with pest behaviour and plant pathogenesis.

The phylum Proteobacteria was the most abundant in the identified microbiota profiles (98–99%). Surprisingly, this result is non-concordant with the previous evidences where phylum Actinobacteria followed by Proteobacteria dominated the bacterial communities associated with *Scirtothrips dorsalis* (Dickey et al., 2014); Proteobacteria (∼57%) followed by Actinobacteria (∼29%) in fungivorous thrips, *Hoplothrips carpathicus* (Kaczmarczyk et al., 2018) and Proteobacteria (∼21%) followed by Firmicutes (∼16.5%) in onion thrips *Thrips tabaci* (Gawande et al., 2019). These variations in the abundance of the phyla may be due to the different habitats of thrips species with different environmental conditions.

G. uzeli feed on *F. benjamina* leaves and sucks its phloem, while *H. carpathicus* feeds on fungus and *T. tabaci* feeds on onion leaves. Thus, completely different diet may be one of the factor that is influencing the abundance of particular phyla present in associated bacterial communities. Further analyses at the genus level showed that *Arsenophonus* contributed about 98–99% of all microbiota pro-

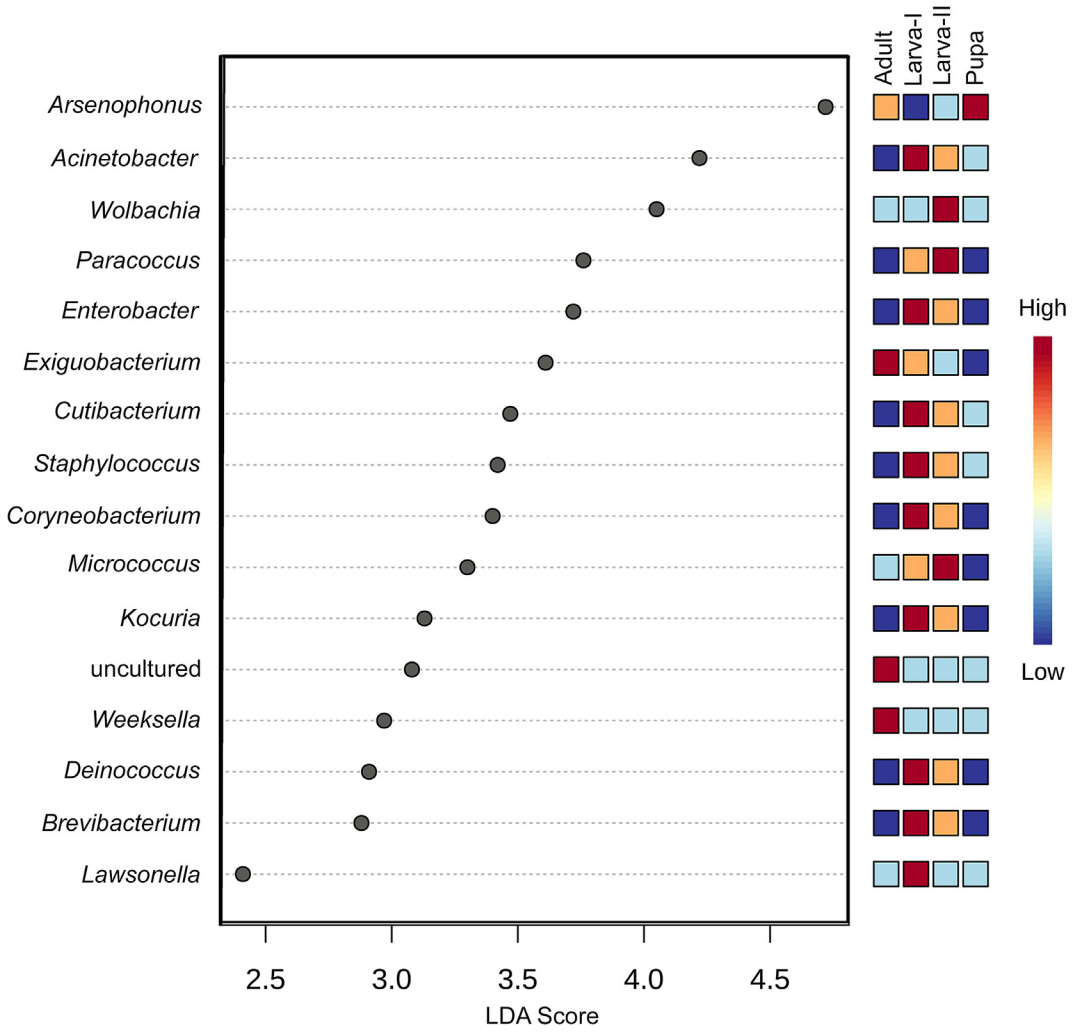

Fig. 3. LEfSe discriminant analysis (LDA) results based on genus level in developmental stages of *G. uzeli*.

files and rest 1–2% abundance was contributed by genera *Acinetobacter*, *Staphylococcus*, *Pseudomonas*, *Paracoccus*, *Coryneobacterium_1*, *Cutibacterium*, *Enterobacter*, *Wolbachia* etc. The findings also were not in agreement with the previous results. For example, in bacterial communities associated with *H. carpathicus,* researchers observed *Agrobacterium*, *Erwinia*, *Methylobacterium*, *Pseudomonas*, *Serratia* etc. as dominant genera (Kaczmarczyk et al., 2018) while in turn, in microbiota of *T. tabaci Streptococcus, Pseudomonas, Rosenbergiella, Alistipes*, and *Saccharopolyspora* were the most abundant (Gawande et al., 2019). Furthermore, the gut bacterial genera abundance in other thrips species was totally different than that of *G. uzeli* (de Vries, et al., 2008; Chanbusarakum and Ullman 2008; de Vries et al., 2012; Gitaitis et al., 2003). The reason of differences in the genera abundance characteristic for *G. uzeli* may be due to the vertical transmission of plant pathogenic genus *Arsenophonus*, which is an internal symbiont. Moreover, the dendrogram analysis using unweighted unifrac analysis (without *Arsenophonus* reads) revealed that microbial profiles of larva-I, larva-II and pupa were more similar to each other than to profiles of adults. It nullified the previous findings where researcher claimed that bacterial diversity structure became more complexed as the developmental stage progresses (Kaczmarczyk et al., 2018) but in our study different trend in the bacterial diversity structure was observed. Based on this, it may be concluded that it is not necessarily true that complexity of bacterial diversity structure increases as the developmental stages progresses but it varies from species to species.

Further, it is known that leaves extract of *F. benjamina* possess great antibacterial, antiviral, antioxidant, antinociceptive and anti-inflammatory activities (Imran et al., 2014; Sirisha et al., 2010). Thus, the significant reason explaining the low bacterial diversity structure may be due to the high anti-bacterial activity of leaves extracts with the presence of phytochemicals such as alkaloids (e.g. isoquinoline, indole, pyridine, carbazol, quinolizidine, indolizidine, pyrrolizidine, quinolone, pyrrolidine, tropane, acridine etc.) along with cinnamic acid, caffeic acid, stigmasterol, triterpenoids, quercetin etc. Previous analyses supported the increased antibacterial activities of those phytochemicals against *S. aureus, E. coli, S. typhi, K. pneumonia, P. aeruginosa, B. cerus, B. subtilis, A. niger*, and *C. albicans* (Imran et al., 2014; Dai et al., 2012; Ashraf et al., 2020). On the other hand, the high abundance of *Arsenophonus* in *G. uzeli* suggest that representatives of this genus may be resistant and/or tolerant to different antibacterial agents, alkaloids, flavonoids, and glycosides (e.g. quercetin).

The predicted metabolic functions of all the developmental stages of *G. uzeli* using PICRUSt2 analysis revealed that bacterial communities were highly involved in biosynthesis of amino acids, phenylalanine, tyrosine, tryptophan, uniquinone and other terpenoid-quinone. In addition, the tested microbiota showed activities towards the metabolism of carbon, nitrogen, amino acids,

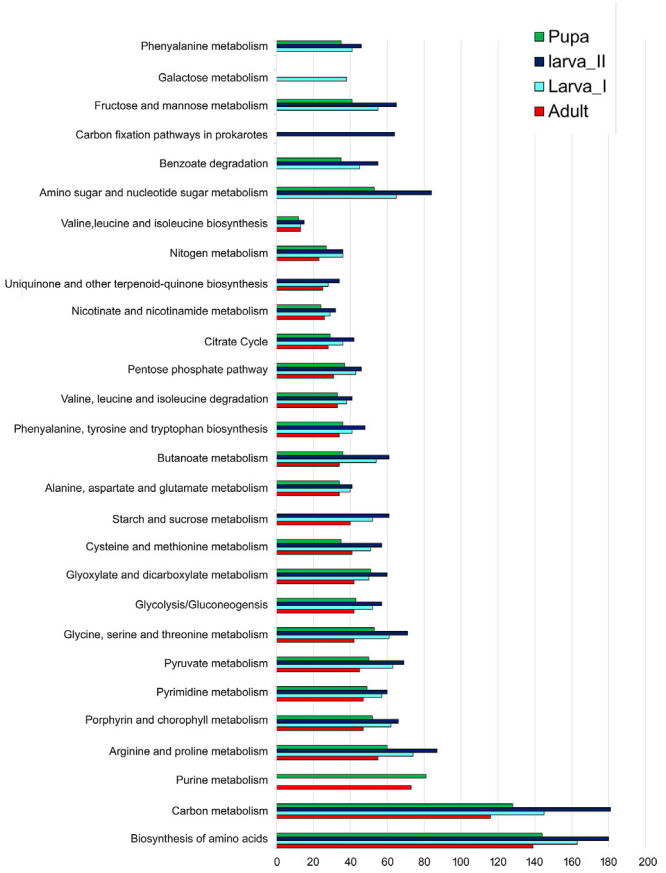

Fig. 4. Representation of the common predicted functional metabolic pathway (PICRUSt2) between developmental stages of the *G. uzeli*.

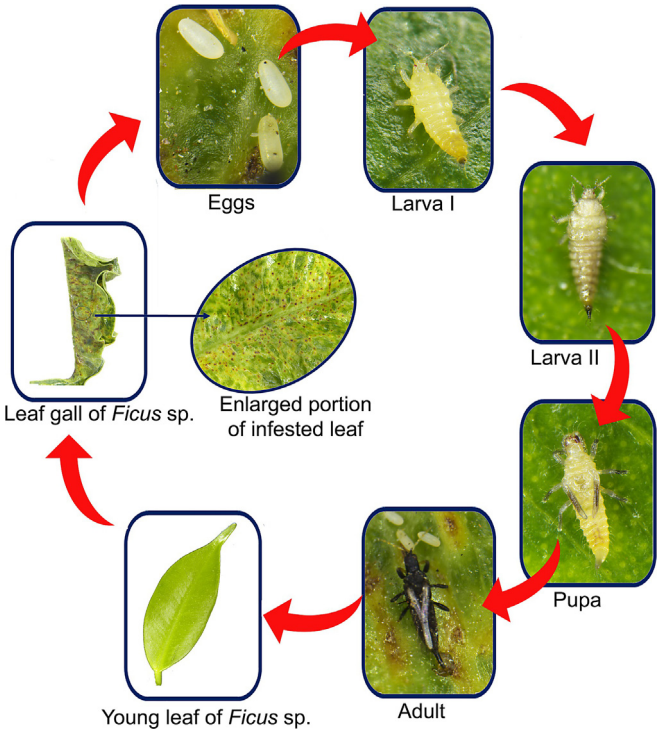

Fig. 5. Schematic presentation of formation of developmental stages in *G. uzeli* along with the gall inducing behaviour plant pathogenesis behaviour.

porphyrins, butanoate etc. along with degradation activity of benzoate and amino acids. Further, the higher relative abundance of predicted genes revealed the presence of higher activity for amino acids biosynthesis and carbon metabolism in all the developmental stages (Fig. 4). The findings were non-concordant with results described by Kaczmarczyk et al., 2018 wherein authors observed different metabolic pathways in *H. cephalicus*. Furthermore, PICRUSt2 results also indicated that the mapped microbial profile may be capable of metabolizing the phytochemicals present in *F. benjamina* leaves through different routes.

An endosymbiosis association of *Arsenophonus* with *G. uzeli* was observed here for the first time. It is concordant with the findings of Dickey et al., 2014, where authors reported the presence of *Arsenophonus* along with *Wolbachia, Rickettsia, Spiroplasma*, and *Cardinium* in *Scirtothrips dorsalis*. Further, they reported the horizontal transmission of these endosymbionts in selected species (Dickey et al., 2014). However, detailed analysis on other thrips species (Wells et al., 2009; Powell et al., 2015; Kaczmarczyk et al., 2018; de Vries et al., 2008; Gawande et al., 2019) indicated the vertical transmission of endosymbionts, as observed in the case of *G. uzeli*. Our findings about genus *Arsenophonus* as son-killer is very interesting as this genus can alter the sex ratio towards females. In our study, only female specimens of *G. uzeli* were observed which may be due to the induced thelytoky (parthenogenesis to produce females from unfertilized eggs) by *Arsenophonus*. Such a phenomenon dependent directly on the presence of *Arsenophonus* has been also described for *Nasonia* species and other hymenopterans. Literary evidences also support thelytoky in *Nasonia* species and other hymenopterans is due to the presence of *Arsenophonus* (Skinner 1985; Werren et al., 1986; Ferree et al., 2008). Nevertheless, a robust sampling of *G. uzeli* from different geographical locations is required to confirm the role of *Arsenophonus* as male-killing endosymbiont promoting thelytokous reproduction of its host.

Besides endosymbiotic association, *Arsenophonus* also mediates the relationship between *G. uzeli* and the host plant *F. benjamina*. *G. uzeli* feeds on leaves and sucks the phloem out. During this process thrips put *Arsenophonus* directly into *F. benjamina* leaves what in the future leads to the formation of galls and patches (Fig. 4). The proposed mechanism for the transfer and phytopathogenecity action of *Arsenophonus* may be as follows: (i) phloem feeding insect (*G. uzeli*) sucks the phloem out and during this process large amount of saliva is secreted to the leaf tissue; (ii) transfer of *Arsenophonus* to leaf tissues through excess saliva secretion; (iii) gall induction and patch formation (leaf infected); (iv) formation of developmental stages of thrips in induced galls. According to proposed mechanism, healthy leaves are infected via introduction of *Arsenophonus* through saliva during phloem sucking by *G. uzeli* adults. This mechanism keeps in view the previously described pathway i.e. circulative propagation of microorganisms (e.g. *Phytoplasma*, *Spiroplasma*, and *Liberibacter*) in insect's bodies and inoculation via saliva during phloem sap-feeding (Orlovskis et al., 2015). Described findings lead in turn to the fact that the transition of obligate/facultative symbiont *Arsenophonus* from thrips to plant (where its act as a pathogen) is a perfect trade-off example between vertical transmission (*G. uzeli*) and horizontal transmission (plant infection). Moreover, evidences of phytopathogenecity of *Arsenophonus* (e.g. marginal chlorosis in strawberries (Zreik et al., 1998), syndrome basses richesses (SBR) in sugar beet and strawberry (Bressan et al., 2012) have been already reported in the literature. Previously described results suggest that *Arsenophonus* was preadapted to acts as phytopathogen and this shift in lifestyle from endosymbiont to plant pathogen could be related to the environmental conditions or transmission of genetic material (e.g. bacteriophages, transposons, and plasmids). The transmission of these genetic material was known to be the major contributors

towards the horizontal gene transfer (HGTs) which in turn allows the microbial diversity to acclimatize to the new environments (Stavrinides 2009).

4. Conclusion

Based on the results obtained, the conclusion drawn was mentioned below:

- Gut microbiota in developmental stages of *G. uzeli* was dominated by *Arsenophonus* (98–99%) and rest 1–2% was contributed by genera like *Acinetobacter*, *Staphylococcus*, *Pseudomonas*, *Paracoccus*, *Coryneobacterium_1*, *Cutibacterium*, *Enterobacter*, *Wolbachia*, etc.
- *Arsenophonus* was transmitted vertically through all the developmental stages of *G. uzeli*.
- *Arsenophonus* in the current dataset was enhancing the thelytoky behaviour and in turn act as son killers for *G. uzeli*.
- Plant pathogen *Arsenophonus* was major contributing factor responsible for galls inducing behaviour of *G. uzeli* in *F. benjamina*.

Furthermore, an extensive effort in terms of *G. uzeli* developmental stages specimen's collection along with the microbial profiles of infected leaves from different geographical locations is required to accurately predict the function of genus Arsenophonus in *G. uzeli*.

5. Availability of data and material

The generated raw reads were submitted under the BioProject ID PRJNA741334 (Accession Number: SAMN19866870-SAMN19866879) to the National Center for Biotechnology Information (NCBI) GenBank Portal.

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CRediT authorship contribution statement

Kaomud Tyagi: Conceptualization, Data curation, Methodology. **Inderjeet Tyagi:** Conceptualization, Data curation, Methodology. **Abhishek Patidar:** . **Devkant Singha:** . **Agnieszka Kaczmarczyk-Ziemba:** . **Dhriti Banerjee:** . **Vikas Kumar:** Conceptualization, Data curation, Methodology, Funding acquisition.

Declaration of Competing Interest

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

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