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Bacterial Diversity of the Silverleaf Whitefly, *Bemisia tabaci* (Gennadius), Collected on Jasmine and Chrysanthemum

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Authors' contributions

This work was carried out in collaboration among all authors. Author KP conceptualized the study and prepared drat of the manuscript. Authors JND and SB conceptualized the study, prepared research proposal and corrected the data. All authors read and approved the final manuscript.

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ABSTRACT

The adults and nymphs of *Bemisia tabaci* were collected on jasmine and chrysanthemum during 2021-2023 from Southern part of Karnataka, India. Bacterial colonies were isolated from adults and nymphs of *B. tabaci* using spread-plate technique and identified through 16srRNA sequencing. The *B. tabaci* collected on jasmine harboured more number of bacteria (8 species) than chrysanthemum (5 species). Most bacterial species isolated from *B. tabaci*, collected on jasmine and chrysanthemum, belonged to the phylum Bacillota (62.5 and 60 percent). Similarly, Bacilli, Bacillales and Bacillaceae were the dominant class, order and family of the bacteria associated with *B. tabaci*. Nymphs harboured higher number of bacteria than adults in both jasmine (62.5 and 33.5 percent, respectively) and chrysanthemum (60 and 40 percent, respectively). *Bacillus cereus*

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and *B. pumilus* were commonly found species from the nymphs and adults of *B. tabaci* on jasmine. When insect hosts shifted, change in the nutrition and defence system of the host plant influences the bacterial diversity of the insect.

Keywords: 16S rRNA; B. tabaci; chrysanthemum andjasmine.

1. INTRODUCTION

Whitefly (Hemiptera: Aleyrodidae) is one of the most economically important groups of pests with global distribution and very wide range of host plants [1]. It causes damage by acting as the vector for various plant viruses (*Begomovirus*, *Crini* virus, *Clostero* virus *etc*.) and by passively encouraging sooty mould deposits on plants through honeydew secretion [2]. The sooty mould formed by the honeydew leads to the closing of stomata and; as a result, it,interrupts and leads to poor development of the plants. The silverleaf whitefly, *Bemisia tabaci* (Gennadius) has originated in Central Asia and invaded major host crops including field crops (Green gram, Soybean, Blackgram), vegetables (Tomato, Chilli, Bhendi, Brinjal, Beans, Gourds), flower crops (Chrysanthemum, Jasmine, Marigold) and commercial crops (Cotton, Tobacco, Jute); in the area its infestation on plantation crops is rarely seen. Currently, there are 40 cryptic species of *B. tabaci* (morphologically indistinguishable but genetically distinct species) recorded globally. The Middle East–Asia Minor 1 (MEAM1) and Mediterranean (MED) complexes (previously known as B biotype and Q biotype, respectively) are considered as the most invasive species with broad host range of plants. New world 1 (NW 1, A biotype) is also reported in some parts of the world [3].

One of the factors for successful establishment of *B. tabaci* is its nutritional flexibility which is favoured by endosymbionts The two main functions of these endosymbionts of sap sucking insects are; those which are beneficial to the insect under specific ecological conditions and those which play a role in metabolic activities of the insect. Gosalbes *et al*., [4]. Along with this, the microbes inside the insects play major role in their survival, development, reproduction, fecundity, viral transmission and resistance of the host insects? against the various chemicals. About 99 percent of symbiotic bacteria are non-culturable under laboratory conditions [5] but advances in molecular biology have outstandingly improved the culture-independent techniques to study microorganisms, all praises to PCR amplification of bacterial genes straight from environmental samples, pursued by direct sequencing of PCR products. Different gene targets like 16S, 23S, and GroEL have been used to identify bacteria. Several studies have used PCR techniques to identify the different endosymbionts like *Portiera aleyrodidarum* (Primary), *Wolbachia*, *Rickettsia, Arsenophonus, Cardinium, Hamiltonella, Fritschea*, *Bacillus*, *Staphylococcus*, *Enterococcus* (Secondary) in *B. tabaci*. The current study is giving special emphasis on the diversity of facultative bacteria in the nymphs and adults of *B. tabaci* on tomato.

2. MATERIALS AND METHODS

The whiteflies and nymphs collected on jasmine and chrysanthemum from different locations of Southern Karnataka, India were starved for 3 h and surface sterilized with 70 per cent ethanol for 1 minute followed by 0.1 per cent sodium hypochlorite for 1 minute and then rinsed with sterile distilled water for 2 to 3 times to remove the external microbes and wax.

2.1 Serial Dilution and Plating

The adults *B. tabaci* (n=10) were surface-sterilized and crushed in a sterilized 1.5 ml micro-centrifuge tube using a sterilized micro pestle with 1 ml of phosphate buffer saline (PBS) solution (pH 7.4). Prior to that, micro-centrifuge tubes were labelled with date, host and location. The homogenized samples were centrifuged at 2000 RPM for 10 minutes. Then, 100 µl of the homogenized mixture was added to micro centrifuge tubes containing 900 µl of sterile distilled water and serial dilution of samples was made up to 10^{-7} dilutions. 100 µl of aliquot of all the dilutions were plated on both 1M of nutrient agar media and spread using a sterilized glass spreader. Petri plates were then incubated at 28 ^{0}C for 24 to 48 h in bio-oxygen demand (BOD) incubator. The plates were observed for microbial growth afterwards every 24 hours.

2.2 Purification and Storage of Colonies of Bacteria

Representative colonies from each bacterial colony showing similar morphology were selected and pure cultures were obtained by sub-culturing them in the same media. The pure

cultures were added to autoclaved nutrient broth in sterilized test tubes along with respective labels and incubated at 28 ℃ for 24 h in BOD until the clear nutrient broth turn into turbid by the multiplication of bacterial cells.

2.3 Bacterial Genomic DNA Isolation and Quantification

Bacterial culture grown in a nutrient broth was used for genomic DNA isolation by following sucrose buffer method. 1.5 ml bacterial culture was transferred to a sterilized micro centrifuge tube with respective label and centrifuged at 1000 rpm for 3 minutes to get a pellet. Later, supernatant was discarded and pellet was retained. It was repeated with a 1.5 ml culture to collect the sufficient amount of pellet. The pellet was re-suspended into 400 µl sucrose buffer (consists of 1M Tris, 0.5M EDTA and 10 per cent sucrose) and subjected to vortex (SPINIX) to dissolve the pellet. Then, 32µl lysozyme was added and incubated for 10 min at 60℃ in hot water bath. 140 µl of freshly prepared 10 per cent sodium dodecyl sulphate (SDS) was added along with 5 µl of protease. Later, 240 µl of NaCl (5M) and freshly prepared 10 per cent CTAB was added and incubated for 10 min at 60 ℃. It was followed by addition of 500 µl chloroform: isoamyl alcohol (24:1) and mixed well by inverting the tube until the phase is mixed completely. The mixture was centrifuged at 12000 rpm in a micro centrifuge (SPINWIN MC03) for 10 min. Upper aqueous phase was transferred to a new labelled tube and 50µl of 3M sodium acetate (ice cold) was added and mixed well. Then 300 µl isopropanol (ice cold) was added and gently mixed to precipitate DNA and the sample was incubated overnight at -20℃.

The sample was spun at 12000 rpm for 15 min on the next day to pellet down DNA and 1ml of 70 percent ethanol was added to the pellet and spinning was done at 12000 rpm for 10 min (twice). Then the supernatant was discarded and the pellet was allowed for air dry. After complete drying, the DNA pellet was re-suspended in 30 µl of protease, DNase, RNase, free water (GeNei ™) followed by 2 µl of RNase treatment and incubation at 60 ℃ in water bath stored at -20 ℃ until use [6]. The concentration of isolated DNA was quantified by using nanodrop.

2.4 Quality and Quantity Check of Genomic DNA

The quality of genomic DNA was checked by 0.8 percent (0.8g in 100 ml) of agarose which was dissolved in 100 ml of 1X TAE buffer in microwave oven and 5 µl EtBr was added after cooling. This mixture was poured into a pre-set template used with appropriate comb kept on the template, to make wells and the gel was allowed for solidification for 45 minutes. After that, 2µl of DNA was loaded with 2 µl of loading dye (6X Cresol-red DNA loading dye). Electrophoresis was carried at 80 V for 45 min. The genomic DNA was visualized on UV transilluminator (Bio-Rad, USA) and documented using gel documentation system (GelDoc Go).

The amplification of 16s rRNA was carried out by using the universal primer (Forward-5'AGAGTTTGATCCTGGCTCAG3' and Reverse-5' ACGGCTACCTTGTTACGACTT - 3'). The stocks of primers were prepared as per the instructions given and prepared a working primers by adding 0.1 ml of stock in 0.9 ml double distilled water, further stored at -20 ℃. Polymerase chain reactions were performed with 25 µl of PCR mixture in PCR system (ProFlex) with an initial denaturation at 94 °C for 3 minutes, followed by 35 cycles each consisting of denaturation for 1 minute at 94°C, annealing for 45 seconds at 59°C with an extension for 1.5 minute at 72°C followed by final extension for 10 minutes at 72 °C and kept hold at 4 ^{0}C for infinite time. The amplified PCR products were sent for nucleotide sequencing to Eurofins Genomics India Pvt. Ltd. Bangalore. The obtained DNA sequences corresponding to the 16S rRNA gene was confirmed using BLAST search in NCBI. The obtained forward and reverse sequences were aligned together using the NCBI alignment tool to obtain a contig sequence.

3. RESULTS AND DISCUSSION

3.1 Molecular Identification of Bacterial Colonies

Amplification of the 16srRNA gene gave a fragment of about 1500bp. The PCR amplified products were sequenced by using Sanger's Dideoxy sequencing method and obtained results were compared with standard GenBank data base by following NCBI BLAST and species were confirmed.

3.2 Host-wise Bacterial Abundance of *B. tabaci* Collected on Jasmine and Chrysanthemum

The *B. tabaci* population was collected from Jasmine harboring the more bacteria 8 species

i.e., *Bacillus zhangzhouensis, Bacillus cereus, Bacillus cereus, Bacillus pumilus, Bacillus pumilus, Serratia marcescens, Curtobacterium luteum* and*Microbacterium proteolyticum* than Chrysanthemum 5 species, *Bacillus pumilus*, *Terribacillus saccharophilus Bacillus licheniformis*, *Enterobacter hormaechei, Klebsiella variicola* (Table 1).

3.3 Comparison of Bacterial Diversity of *B. tabaci* Collected on Jasmine and Chrysanthemum at Phylum Level

The phylum Bacillota accounted for 62.5 percent and 60 percent of the bacterial population in *B. tabaci* collected on Jasmine and Chrysanthemum, respectively. Actinomycetota was found only in *B. tabaci* collected on Jasmine with 25 percent abundance. Whereas, Pseudomonadota was found with 12.5 percent in *B. tabaci* collected on Jasmine and 40 percent in *B. tabaci* collected on Chrysanthemum (Fig. 1a and 2 a).

3.4 Comparison of Bacterial Diversity of *B. tabaci* Collected on Jasmine and Chrysanthemum at Class Level

The class Bacilli was found dominant in *B. tabaci* (Fig. 1b and 2b). Bacilli from *B. tabaci* collected on jasmine or on chrysanthemum contributed to at least 60 percent of bacterial diversity. Gamma-proteobacteria accounted for, respectively, 40 percent (in *B. tabaci* collected on chrysanthemum) and 12.5 percent (in *B. tabaci* collected on jasmine) of the diversity. 25 percent of the bacterial diversity was contributed to by the class Actinomycetes or Actinomycetota that were collected from *B. tabaci* on jasmine.

3.5 Comparison of Bacterial Diversity of *B. tabaci* Collected on Jasmine and Chrysanthemum at Order Level

In both, *B. tabaci* collected on jasmine and chrysanthemum, the order Bacillales was found dominant (62.5 and 60 percent, respectively). Followed by Enterobacteriales in Chrysanthemum (40 percent), Micrococcales in Jasmine (25 per cent). The order Micrococcales observed only in *B. tabaci* collected on jasmine and Enterobacteriales accounted for only 12.5 per cent in jasmine (Fig. 1c and 2c).

3.6 Comparison of Bacterial Diversity of *B. tabaci* Collected on Jasmine and Chrysanthemum at Family Level

The family, Bacillaceae was recorded abundant in *B. tabaci* collected on jasmine and chrysanthemum (62.5 and 60 per cent), respectively. The family Yersiniaceae and Microbacteriaceae were found only on jasmine with 12.5 and 25 per cent abundance respectively. On chrysanthemum, Enterobacteriaceae was found exclusively and accounted for 40 per cent (Fig. 1d and 2d).

3.7 Comparison of Bacterial Diversity of *B. tabaci* Collected on Jasmine and Chrysanthemum at Genus and Species Level

The genus Bacillus, was found dominant on both jasmine and chrysanthemum. Overall bacterial species diversity revealed that, nymphs harbored more bacteria than adults. Where, nymphs of *B. tabaci* collected on jasmine had 62.50 per cent of the bacteria and adults had 37.50 per cent (Fig.1e). In chrysanthemum, 60 percent of the bacteria were recorded from nymphs and 40 percent from nymphs (Fig. 2e). The bacterial species, *Bacillus cereus* and *B. pumilus* were found common among adults and nymphs of *B. tabaci* collected on jasmine (Fig. 3). Whereas, in *B. tabaci* collected on chrysanthemum, no such commonality was observed (Fig. 4).

The diversity of microbes in the insects varies with hosts on which it feeds, habitat, the pressure of biotic and abiotic factors [7]. In the present study, the bacterial diversity of *B. tabaci* varied with hosts. Change in the diversity of bacteria was observed by Pujar *et al*. [8] in rugose spiraling whitefly, *Aleurodicus rugioperculatus* collected on coconut, banana, maize and arecanut. In the present study, *B. tabaci* collected on jasmine and chrysanthemum showed high dominance of Bacillota, Bacilli, Bacillales and Bacillaceae. As the *Bacillus* sp. helps in the production of medium-length sugars from derived sucrose and increase the stickiness of honeydew [3]. This can be reason for the abundance of Bacillus sp. in *B. tabaci*. These results are in accordance with the report of Pujar *et al*. [9] who recorded bacterial diversity of *B. tabaci* on tomato. Variation in the bacterial diversity in the *B. tabaci* due to change in the host nutrition and defense posed by the host plant against insect. Host plants have a positive impact on the shaping of microbial

communities associated with *Spodoptera littoralis* [10] *Helicoverpa* spp. [11,10,12] *Lymantria dispar* Broderick *et al*., [13] Mason and Raffa, [14] and *Leptinotarsa decemlineata* [15]. Plant characters such as leaf surface, wax composition and the availability of sugars in plants might influence bacterial community composition in the host insect [16].

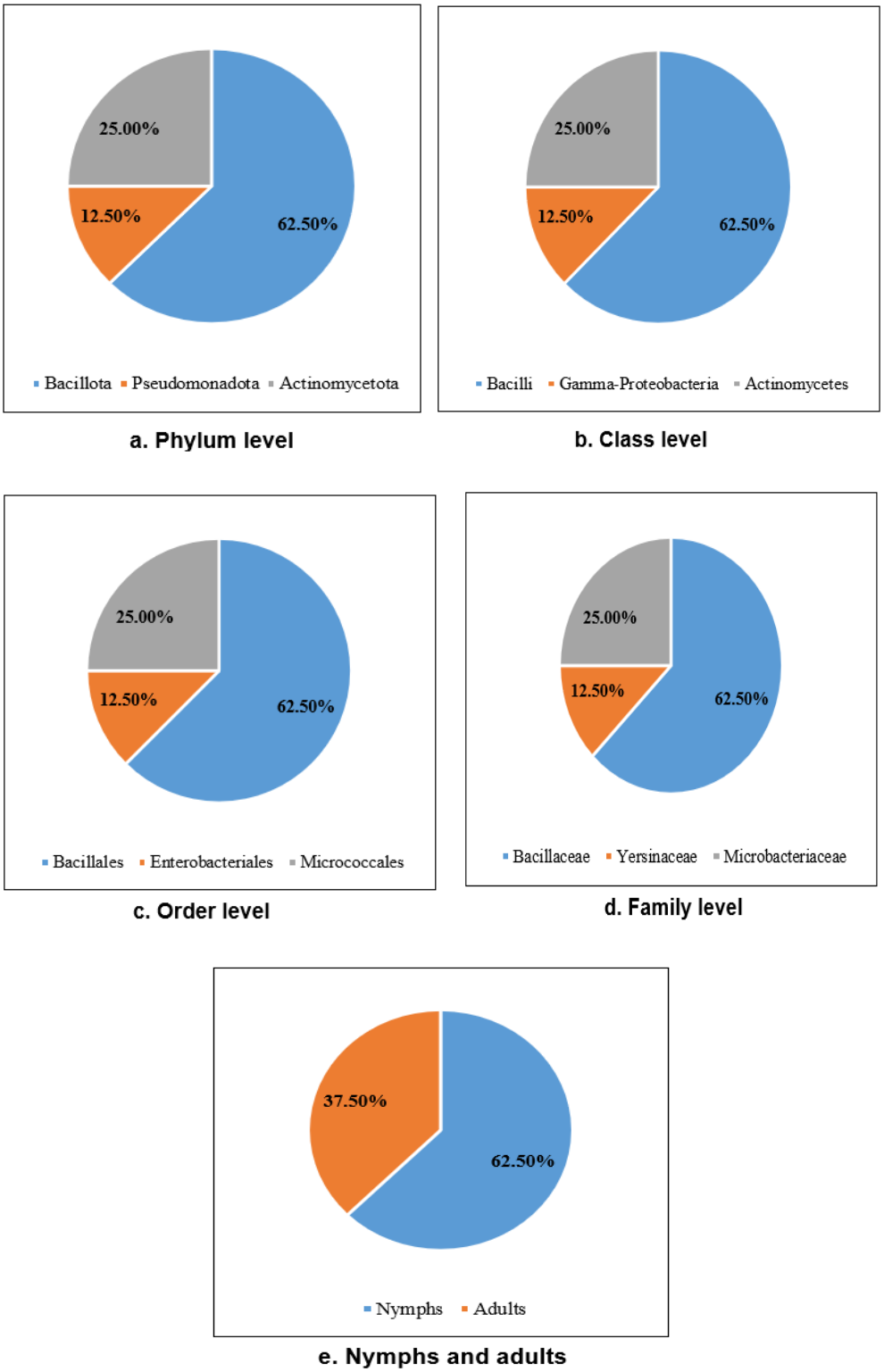

Fig. 1. Diversity of bacteria in B. tabaci collected on Jasmine crop

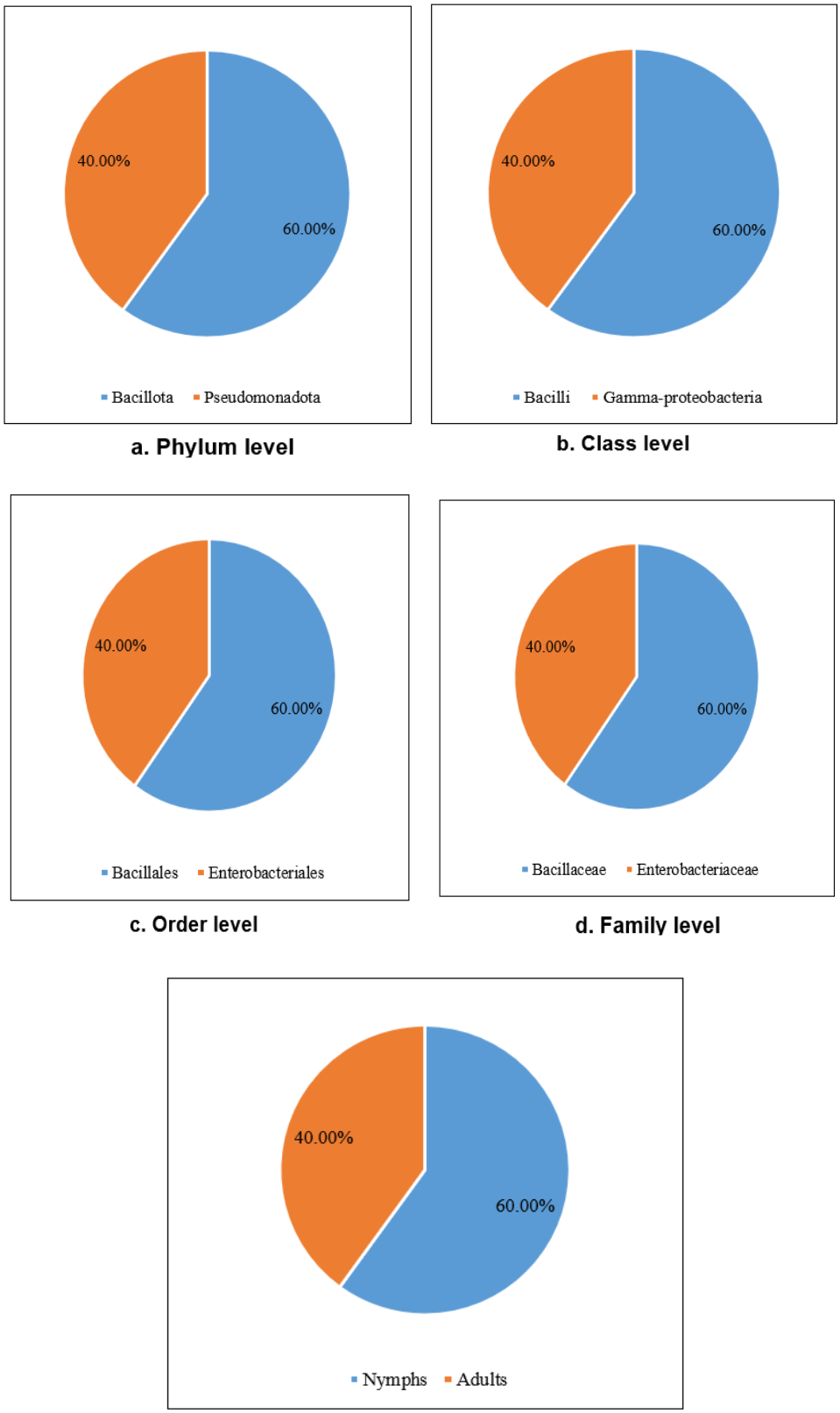

Fig. 2. Diversity of bacteria in B. tabaci collected on chrysanthemum crop

Table 1. Bacterial diversity of *B. tabaci* collected on Jasmine and Chrysanthemum

	Species of bacteria	Jasmine		Chrysanthemum		Family	Order	Class	Phyllum
1	*Bacillus cereus*	Nymph	Adult	-	-	Bacillaceae	Bacillales	Bacilli	Bacillota
2	*Bacillus licheniformis*	-	-	Nymph	-	Bacillaceae	Bacillales	Bacilli	Bacillota
3	*Bacillus pumilus*	Nymph	Adult	Nymph	-	Bacillaceae	Bacillales	Bacilli	Bacillota
4	*Bacillus zhangzhouensis*	Nymph	-	-	-	Bacillaceae	Bacillales	Bacilli	Bacillota
5	*Curtobacterium luteum*	Nymph	-	-	-	Microbacteriaceae	Micrococcales	Actinomycetes	Actinomycetota
6	*Enterobacter hormaechei*	-	-	-	Adult	Enterobacteriaceae	Enterobacteriales	Gamma- Proteobacteria	Pseudomonadot
7	*Klebsiella variicola*	-	-	-	Adult	Enterobacteriaceae	Enterobacteriales	Gamma-Proteobacteria	a
8	*Microbacterium proteolyticum*	-	Adult	-	-	Enterobacteriaceae	Enterobacteriales	Gamma-Proteobacteria	Pseudomonadot
9	*Serratia marcescens*	Nymph	-	-	-	Yersiniaceae	Enterobacteriales	Gamma-Proteobacteria	a
10	*Terribacillus saccharophilus*	-	-	Nymph	-	Bacillaceae	Bacillales	Bacilli	Bacillota

Notes: Nymph= B. tabaci nymph, Adult= B. tabaci adult

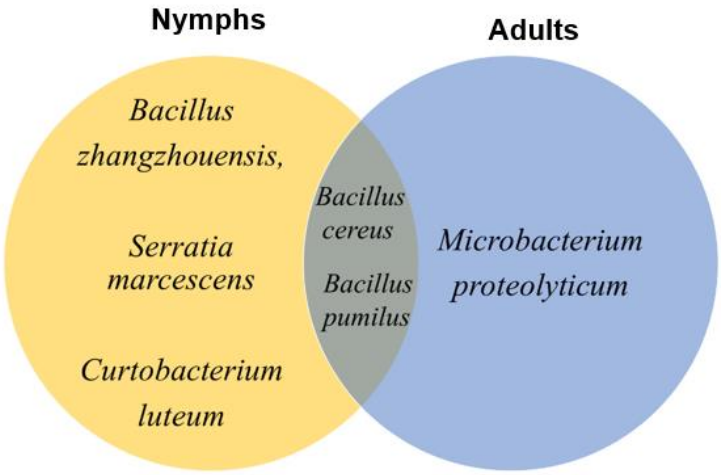

Fig. 3. Bacterial diversity in adults and nymphs of B. tabaci collected on jasmine

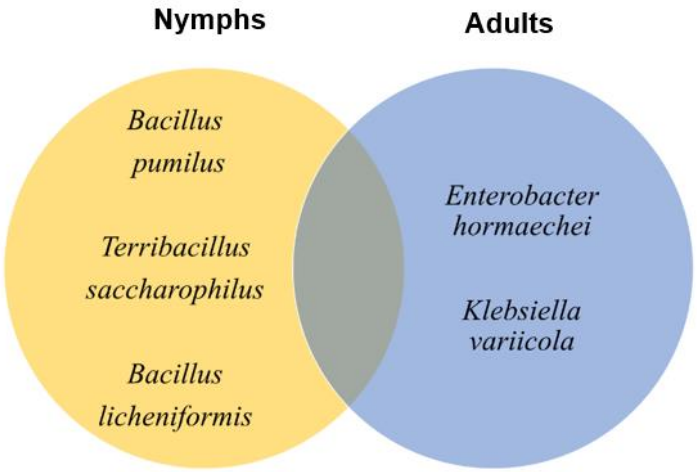

Fig. 4. Bacterial diversity in adults and nymphs of B. tabaci collected on chrysanthemum

4. CONCLUSION

This study presents better understanding of the bacteria associated with *B. tabaci* on jasmine and chrysanthemum. The change in bacterial diversity in different host crops have made a channel to study about how the bacteria are obtained from different crops and eliminated from the insets' body. Understanding the specific functions of each bacteria and the transmission patterns will be a suitable area for future research.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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