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Facultative Bacterial Diversity Associated with Silverleaf Whitefly, *Bemisia tabaci* (Gennadius) on Tomato Crop

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Authors' contributions

This work was carried out in collaboration among all authors. Author SB conceptualized the study. Author KP executed field work, lab experiments, data collection and prepared the manuscript. Author JND did the data analysis and interpretation. All authors read and approved the final manuscript.

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ABSTRACT

The adults and nymphs of *Bemisia tabaci* were collected on tomato crop from different locations of Karnataka during 2021-2023. Bacterial colonies were isolated from adults and nymphs of *B. tabaci* using spread-plate technique and identified through 16srRNA sequencing. Nymphs showed high (55%) abundance of bacteria than adults (45%). 63.64% of the bacterial population in the nymphs belong to the phylum Bacillota followed by pseudomonadota (36.36%). In adults, Bacillota found dominant (100%). The class Bacilli was dominant in both nymphs and adults (63.64 and 100% respectively). In the nymphal stage, Bacillales was dominant order (54.55%). Similarly, in adults also Bacillales was found dominant (77.79%). Bacillaceae was abundant in nymphs (45.45%)and in adults same family accounted for 66.67%. The genus Bacillus was dominant in both nymphs

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(45.45%) and adults (55.55%). The species, *B. licheniformis*, *B. pumilus*, *B. safensis* and *Staphylococcus saprophyticus* were found common between adults and nymphs. The bacterial diversity varies with the different stages of the *B. tabaci* on same host.

Keywords: 16srRNA; bacillus; Bemisia tabaci and facultative bacteria.

1. INTRODUCTION

Whitefly (Homoptera: 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 in an active way by acting as vector for various plant viruses (*Begomovirus*, *Crini*virus, *Clostero*virus *etc*.) and passively by encouraging sooty mould deposits on plants through honeydew secretion [2]. Later, the sooty mould formed by the honeydew secreted by them leads to the closing of stomata as a result the gas exchange by the plants will be interrupted and leads to poor development of plants. So far, 440 species of whiteflies under 63 genera are known from India and few of them are economically important [3]. More than 320 plant species belonging to 225 genera and 73 families in India have been recorded as hosts of whiteflies [4].

The most commonly known whitefly is the silverleaf whitefly, *Bemisia tabaci* (Gennadius), which is originated in Central Asia and invaded all over the world. The major host crops of this pests are field crops (Green gram, Soybean, Blackgram, etc.), vegetables (Tomato, Chilli, Bhendi, Brinjal, Beans, Gourds, etc.), flower crops (Chrysanthemum, Jasmine, Marigold etc.) and commercial crops (Cotton, Tobacco, Jute etc.) and infestation on plantation crops is rarely seen. Currently there are 40 cryptic species (morphologically indistinguishable but genetically distinct in biological characteristic species) has been recorded in *B. tabaci*. 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 [5].

One of the factors for successful establishment of *B. tabaci* is its nutritional flexibility. 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*., [6]. Along with this, the microbes inside the insects plays major role in their survival, development, reproduction, fecundity, viral transmission and resistance against the various chemicals. About 99 per cent of symbiotic bacteria are non-culturable under laboratory conditions [7] 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, GroEL *etc*., have been used to identify bacteria. Several studies have used PCR techniques to identify the different endosymbionts like *Portieraaleyrodidarum* (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 tomato from different locations 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 surface sterilized adults were 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. Then, Petri plates were incubated at 28 ^{0}C for 24 to 48 h in bio-oxygen demand (BOD) incubator. Further, plates were observed for microbial growth after every 24 hours.

2.2 Purification and Storage of Colonies of Bacteria

Representative colony from each colonies showing similar morphology were selected and pure culture was obtained by sub-culturing it 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 400µL sucrose buffer (consists of 1M Tris, 0.5M EDTA and 10 per cent sucrose) and subjected to vortex (SPINIX) to dissolve the pellet. Then, 32 µL 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 50 µL 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 1 mL of 70 per cent 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 [8]. The concentration of isolated DNA was quantified by using nanodrop.

2.4 Quality and Quantity Check of Genomic DNA

Quality of genomic DNA was checked by 0.8 per cent (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

Twenty bacterial isolates were recorded from nymphs and adults of *B. tabaci*, in which nymphs had more number of isolates (11 species) compared to adults (9 species) (Table 1 and 2). The bacterial abundance was observed to be high (55 per cent) in nymphs compared to adults (45 per cent) (Fig. 1).

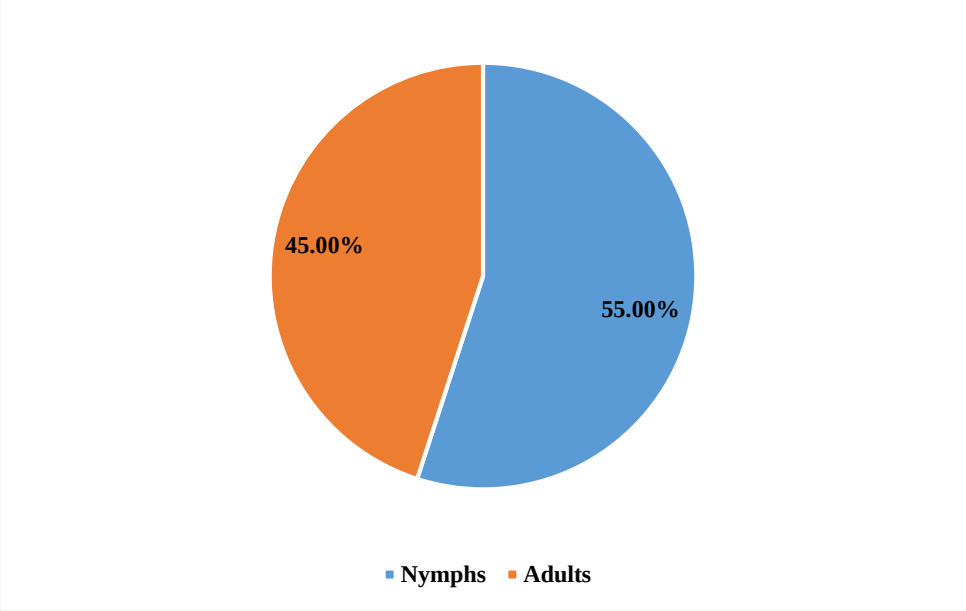

Fig. 1. Bacterial abundance of nymphs and adults of *B. tabaci*

Table 1. Bacterial diversity in the nymphs of the *Bemisia tabaci* collected on tomato crop

Phylum	Class	Order	Family	Species
Bacillota	Bacilli	Bacillales	*Bacillaceae*	*Bacillus licheniformis*
				Bacillus subtilis
				Bacillus cereus
				Bacillus safensis
				Bacillus pumilus
			Staphylococcaceae	*Staphylococcus saprophyticus*
		Lactobacillales	Enterococcaceae	*Enterococcus casseliflavus*
		Enterobacteriales	Enterobacteriaceae	*Klebsiella variicola*
				Atlantibactersubterranea
	Gamma-Proteobacteria		Morganellaceae	*Proteus penneri*
Pseudomonadota		Pseudomonadales	Pseudomonadaceae	*Pseudomonas putida*

Table 2. Bacterial diversity in the adults of the *Bemisia tabaci* collected on tomato crop

Phylum	Class	Order	Family	Adults (9)
Bacillota	Bacilli	Bacillales	*Bacillaceae*	*Bacillus safensis*
				Bacillus aerius
				Bacillus pumilus
				B. licheniformis
				Heyndrickxia oleronia
				Bacillus velezensis
			Staphylococcaceae	*Staphylococcus saproph yticus*
		Lactobacillales	Enterococcaceae	*Enterococcus gallinarum*
				Enterococcus mundtii

3.1 Phylum Level

The bacteria isolated from *B. tabaci* which were collected on tomato grouped into two phyla i.e. Bacillota and Pseudomonadota. 63.64 per cent of the bacterial population in the nymphs belong to the phylum Bacillota and 36.36 per cent belongs to Pseudomonadota. Whereas, in adults, all the obtained bacterial colonies (100 per cent) belong to the phylum Bacillota (Fig. 1).

3.2 Class Level

The class Bacilli found dominant (63.64 per cent) in the nymphs of *B. tabaci* followed by Gamma proteobacteria which accounted for 36.36 per cent. In the adults of *B. tabaci,* all the bacterial colonies (100 per cent) were belong to the class Bacilli (Fig. 2).

3.3 Order Level

The bacterial population of *B. tabaci* collected from tomato were classified into four orders (Bacillales, Lactobacillales, Enterobacteriales and Pseudomonadales). The order Bacillales was found to be the most abundant (54.55 per cent) in the nymphs of *B. tabaci* followed by Enterobacteriales (27.27 per cent). Both Lactobacillales and Pseudomonadales were found least abundant (9.09 per cent each). Whereas, in adults, only two bacterial orders were recorded. Among them, Bacillales was dominant (77.78 per cent) over Lactobacillales which accounted for only 22.22 per cent (Fig. 3).

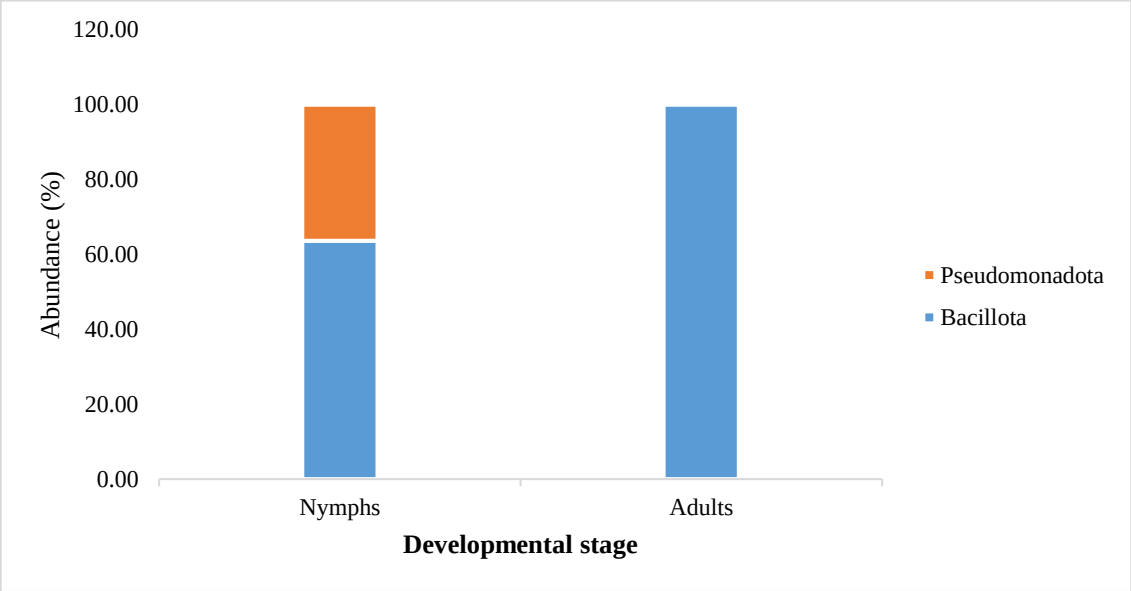

Fig. 2. Bacterial abundance of nymphs and adults of *B. tabaci* at phylum level

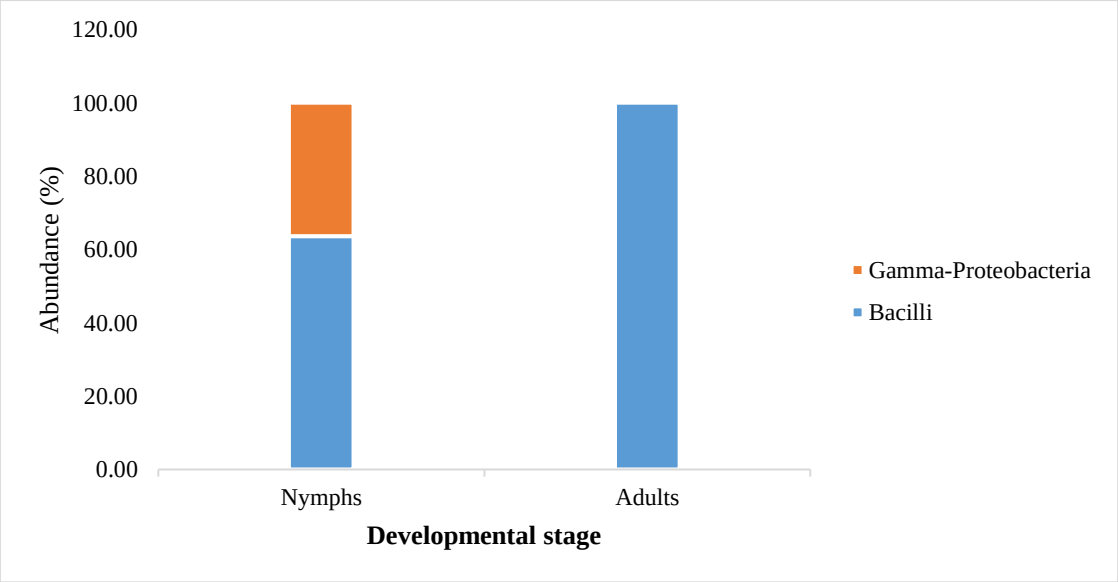

Fig. 3. Bacterial abundance of nymphs and adults of *B. tabaci* at class level

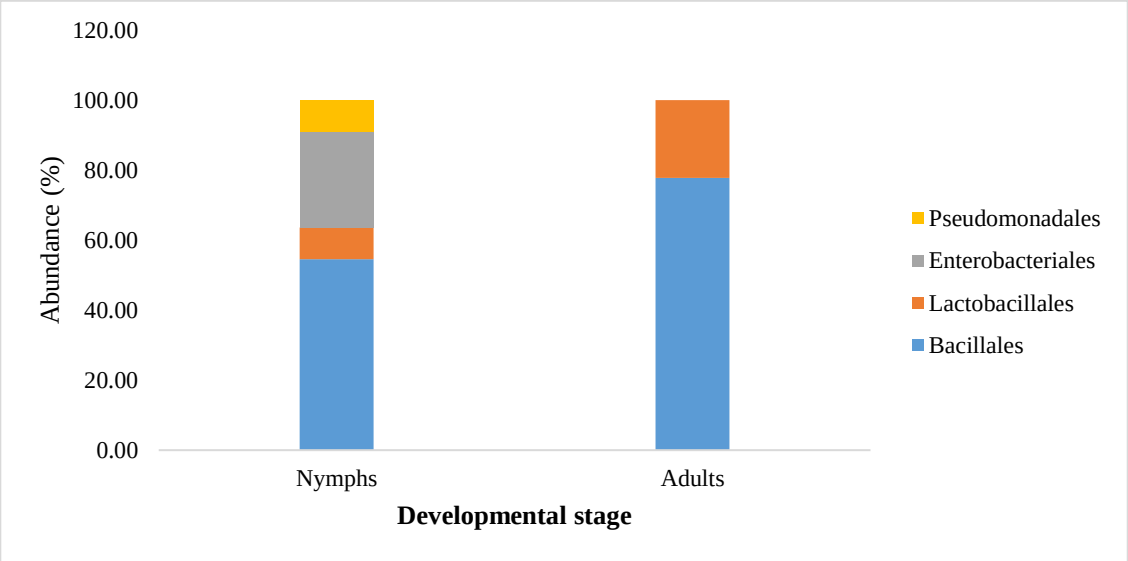

Fig. 4. Bacterial abundance of nymphs and adults of *B. tabaci* at order level

3.4 Family Level

The nymphal stage recorded more number (six families) of bacterial families than the adult stage (three families). In nymphs, majority of the bacterial population was belong to the family, Bacillaceae (45.45 per cent) followed by Enterobacteriaceae (18.18 per cent) and remaining all four families showed equal abundance (9.09 per cent). In case of adults, among the three families, Bacillaceae accounted for 66.67 per cent followed by Enterococcaceae (22.22 per cent) and least abundant family was staphylococcaceae with 11.11 per cent abundance.

3.5 Genus Level

The bacterial population in the nymphal stage of *B. tabaci* distributed under seven different genera. In which, Bacillus was the predominant genus (45.45 per cent) and remaining six genera accounts for 9.09 per cent each. Whereas, in adults, only four bacterial genera were recorded, among them Bacillus was the dominant genus (55.55 per cent) followed by Enterococcus (22.22 per cent). The genera *Klebsiella, Atlantibacter, Proteus Pseudomonas,* were found only in nymphal stage, whereas, the genus *Heyndrickxia*was confined only to the adult stage (Fig. 5).

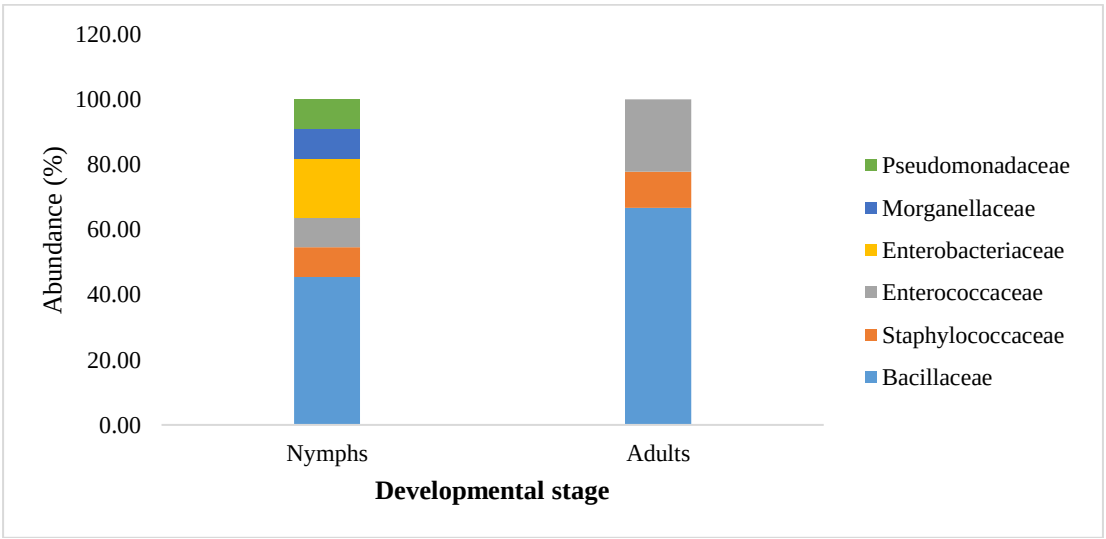

Fig. 5. Bacterial abundance of nymphs and adults of *B. tabaci* at family level

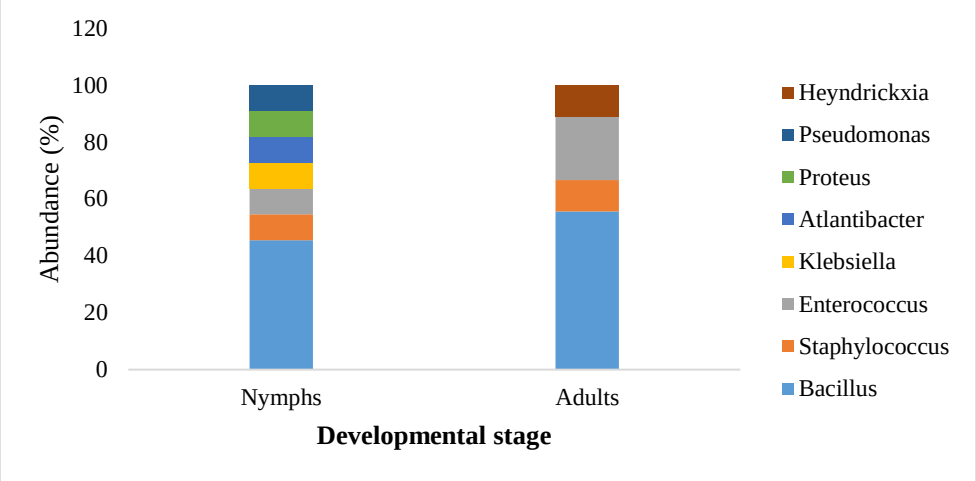

Fig. 6. Bacterial abundance of nymphs and adults of *B. tabaci* at genus level

3.6 Species Level

In the nymphal stage of *B. tabaci*, 11 species of bacteria were recorded whereas, in adults nine bacterial species were recorded. Among them, the bacteria *Bacillus licheniformis*, *B. pumilus*, *B. safensis* and *Staphylococcus saprophyticus* were found common in nymphs and adults (Table 1 and 2).

The endosymbionts of cryptic species of *B. tabaci* was determined by Marubayashi *et al*. [9] through sequencing of 16SrRNA gene. The results of the present study are in accordance with the study of El *et al*. [10] and Li *et al*. [11] who determined the diversity of facultative bacteria *Bacillus*, *Staphylococcus*, *Enterobacater*, *Paracoccus*, *Acinetobacter* through plating techniques. Similarly, twenty different bacterial genera, including 31 species belong to Actinobacteria, 'alpha'-, 'beta'-, 'gamma'- Proteobacteria, and Firmicutes were isolated by Indiragandhi *et al*. [12] from both the B and Q biotypes of *B. tabaci* and among them, *Bacillus*, *Kocuria*, *Moraxellla*, *Micrococcus*, *Sphingomonas* and *Staphylococcus* were common. Moreover, B biotype was associated with *Acinetobacter*, *Deinococcus*, *Modestobacter*, *Microbacterium*, and *Pseudomonas*, whereas, Q biotype was associated with *Arthrobacter*, *Bradyrhizobium*, *Janibacter, Morganella*, *Naxibacter* and *Streptomyces*. Host plants have a great influence on the gut microbial diversity in host insects [13]. The genera Bacillus (30%), Acinetobacter (10%) and Exiguobacterium (10%) were observed by using culture-dependent method by Saranya *et al*. [14] in the rugose spiralling whitefly, *A. rugioperculatus* reared on coconut plants whereas, Bacillus (81%), Lysinibacillus (11%), Arthrobacter (4%) and Pseudomonas (4%) from banana plants. Similarly, Pujar *et al*. [15] recorded the dominance of the phylum Bacillota, class Bacilli, order Bacillales, family Bacillaceae and genus Bacillus in *A. rugioperculatus* collected from four different hosts.

The residing bacteria (not all) perform various functions *viz*., *Bacillus* sp. and *Staphylococcus* sp. produce amylase enzyme which helps in production of medium-length sugars from derived sucrose and increase the stickiness of honeyde [16]. In *A. rugioperculatus*, along with honeydew secretion, *Bacillus* sp. helps in lactose fermentation and siderophore production. In case of termites it involves in cellulose digestion [17] and it helps in detoxification of profenophos and chlorpyrifos in *Paracoccus0 marginatus* [18].

4. CONCLUSION

The symbiotic relationship between the insects and bacteria caused them to be successful creatures on the earth. Though, all the facultative microbes residing in the insects doesn't have any role (called opportunistic microbes) in the insects, some of the microbes are directly or indirectly involving in their survival, reproduction and development of the insects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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