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Diversity of Associated Endosymbionts of *Bemisia tabaci* (Gennadius) on Solanaceous Host Plants in India

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Abstract

Background: Investigations were carried out to record the spreading frequency of seven known endosymbionts specifically *Portiera, Cardinium, Rickettsia, Fritschea, Wolbachia, Hamiltonella and Arsenophonus* between the field populations of *Bemisia tabaci* collected from two host plants, viz. brinjal (*Solanum melongena*) and tomato (*S. lycopersicum*). **Methods:** Individual flies from each host were scanned for symbiotic bacterial infection with specific primers amplifying the 16S rRNA gene of *Portiera, Cardinium, Hamiltonella, Rickettsia* and *Wolbachia,* and the 23S rRNA gene of *Arsenophonus* and *Fritschea*. **Findings:** The primary endosymbiont, *Portiera aleyrodidarum* remained present in the samples of *B. tabaci* on both host plants brinjal and tomato but a variation was observed in the distribution frequency of secondary endosymbionts. **Application:** This irregular distribution of secondary endosymbionts strengthens the hypothesis that each endosymbiotic bacterium not only has a role in the survival but may also have a part in the polyphagus nature of *B. tabaci*.

Keywords: *Bemisia tabaci*, Distribution, Diversity, Endosymbionts, Genetic Group

1. Introduction

Bemisia tabaci (Gennadius) (Hemiptera:Aleyrodidae) is a globally known polyphagous pest reported in over 900 host plants including vegetables, ornamentals, other agricultural and horticultural crops[1,2]. *B. tabaci* is a cryptic species complex of minimum 24 genetically diverse species. Thus far, one obligate and seven facultative symbiotic bacteria have been described from the *B. tabaci* species complex. Both genetic groups and infested symbionts are very significant to evaluating the pest rank of *B. tabaci*[3]. This pest features on the list covering world's 100 destructive species of International Union for Conservation of Nature and Natural Resource[4]. It causes economic damage as a pest in over 60 crop plants acting as a vector of many plant viruses, e.g. Mungbean Yellow Mosaic Virus (MYMV) is one of the most common diseases of Mungbean and is transmitted through whitefly *B. tabaci*[5]. This sap sucking pest feeds on phloem sap enriched in carbohydrates and is unable to get crucial amino acids and nutrients that are required for its growth and development. This deficiency is compensated by the microbial community in the form of endosymbionts, which are either primary endosymbionts or secondary endosymbionts. Of these, the Primary or obligatory endosymbionts (P-endosymbionts) reside in bacteriocytes[6]. These are transferred vertically and are identified to produce important non-dietary metabolites[7,8]. The secondary endosymbionts also transferred vertically but are described to be communicated horizontally over direct or indirect interaction with other infected individuals[7,9,10]. *Portiera aleyrodidarum* is notable as the only primary or obligate endosymbiont of whitefly[11]. But a number of secondary endosymbionts like *Wolbachia* (Rickettsiales)[12], *Arsenophonus* (Enterobacteriales)[13], *Cardinium* (Bacteroidetes)[14], *Rickettsia* (Rickettsiales)[15], *Hamiltonella*

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(Enterobacteriales)[12] and *Fritschea* (Chlamydiales)[16] are known.

The secondary endosymbionts are known to have lot of impact on the whiteflies, such as heat tolerance[17], resistivity to parasitoids[18], capability of virus transmission[19] and vulnerability to insecticides[20,21]. Infection of *Rickettsia* is reported to increase fitness substantially and female bias in the host population[22]. *Wolbachia* has ability to array host reproduction, deliver nutrition to insect hosts and shield insect hosts from pathogenic viruses[23]. The symbiont in this condition acts as equally mutualist and multiplicative manipulator for the host insect, with obvious helpful impact on host population increase as well as the spread of symbiont in fields. According to[24], some secondary endosymbionts may even deploy interbreeding among whitefly species. Strains of endosymbiotic bacteria infecting whiteflies have been described to intermingle precisely with diverse whitefly populations with wide-ranging effects on its host biology and effectiveness of virus transmission[25].

Although case studies on the role of secondary endosymbionts in *B. tabaci* are very limited, these bring out their wide-ranging impact on the biology of their hosts. Thus knowledge of diversity of Secondary endosymbionts (S-endosymbionts) is essential for clarifying various ecological aspects of *B. tabaci* species complex.

In this study, we explored the seven known endosymbionts, namely *Portiera*, *Cardinium, Rickettsia, Wolbachia, Fritschea, Hamiltonella* and *Arsenophonus* between the field populations of *B. tabaci* species complex infesting two solanaceous hosts *viz*. brinjal (*Solanum melongena*) and tomato (*Solanum lycopersicum*). The main objective was to generate information on the distribution frequency of the S-endosymbionts.

2. Material and Methods

2.1 Sample Collection

Bemisia tabaci species complex used in the present study were caught from fields of Indian Agricultural Research Institute, New Delhi, during 2015 and 2016 and stored individually in eppendorf tubes comprising 100% ethanol and kept at -20^{0}C until processed for genomic DNA isolation. A total of 30 individuals from each host were processed as samples.

2.2 DNA Extraction

Each individual fly was washed twice by sterile distilled water and total genomic DNA was isolated by using DNASure Tissue Mini Kit (Nucleo-pore, Genetix) as per manufacturer's protocol. The isolated genomic DNA of each replicate was stored at -20°C for further processing.

2.3 Identification of *B. tabaci* Genetic Groups

Molecular characterization of *B. tabaci* for identification of the genetic group was performed based on mitochondrial cytochrome oxidase 1 (mtCO1) subsequently PCR reaction using universal primers[26] (Table 1). PCR was completed with a concluding volume of 25 µl consisting of Thermo Scientific maxima hot start PCR master mix (12.5 µl), molecular grade water (8.5 µl), forward and reverse primers (10 pmol each 1 µl) and 2 µl of genomic DNA. Samples were amplified using a Ventri® 96-well thermal cycler (Applied Biosystems® Life Technologies). The PCR program for amplification of mtCO1 is given in Table 2. The products (5 µl) were visualized in 1% agarose gel containing ethidium bromide under UV illumination after a passage of 45 minute at 80 V. With the expected band size (Table 1) on the gels, the product (20 µl) was used for sequencing.

Databases for sequences were searched using the BLAST algorithm[27,28] in NCBI Gene Bank (NCBI) and were aligned using BioEdit version 7.2.5[29]. Distance was considered using the Kimura 2-parameter model of MEGA 6.

2.4 Screening and Identification of S-endosymbionts

Individual flies from each host were scanned for endosymbiotic bacterial infection with specific primers amplifying the 16S rRNA gene for *Rickettsia, Cardinium, Hamiltonella* and *Wolbachia* and the 23S rRNA gene for *Fritschea* and *Arsenophonus* (Table 1). The existence of primary symbiont *Portiera* was correspondingly checked to approve the quality of DNA extraction. DNA was amplified in a concluding volume of 25 µl, containing 12.5 µl Thermo Scientific maxima hot start PCR master mix, 8.5 µl molecular grade water, 1 µl of both forward and reverse primers (10 pmol each) and 2 µl genomic DNA. The PCR program for the primary and S-endosymbionts is given in Table 2.

Table 1. PCR primers and conditions used in the study

Targeted gene	Primer's Sequence (5'-› 3')	Annealing temp. (^{0}c)/ Product size (bp)	Reference
Portiera 16S rRNA	F- CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCC CCCGCCCG R- CCGTCAATTCMTTTGAGTTT	60/ 550	42
Hamiltonella 16S rRNA	F- TGAGTAAAGTCTGGAATCTGG R- AGTTCAAGACCGCAACCTC	60/ 700	10
Wolbachia 16S rRNA	F- CGGGGGAAAAATTTATTGCT R- AGCTGTAATACAGAAAGTAAA	55/ 700	43
Arsenophonus 23S rRNA	F- CGTTTGATGAATTCATAGTCAAA R- GGTCCTCCAGTTAGTGTTACCCAAC	60/ 600	11
Cardinium 16S rRNA	F- GCGGTGTAAAATGAGCGTG R- ACCTMTTCTTAACTCAAGCCT	58/ 400	12
Rickettsia 16S rRNA	F- GCTCAGAACGAACGCTATC R- GAAGGAAAGCATCTCTGC	60/ 900	13
Fritschea 23S rRNA	F- TGGTCCAATAAGTGATGAAGAAAC R- GCTCGCGTACCACTTTAAATGGCG	60/ 600	44
B. tabaci MtCOI	F- TTGATTTTTTGGTCATCCAGAAGT R- TCCAATGCACTAATCTGCCATATTA	52/ 800	22

Table 2. PCR programs used to detect the prevalence of Primary and Secondary endosymbionts in *B. tabaci*

Endosymbionts	Pre- denaturation	Denaturation	Cycling conditions		
			Annealing	Extension	Cycles
Portiera	94 ^{0}C (4 Min)	94 ^{0}C (30 s)	56 ^{0}C (2 Min)	72 ^{0}C (2 Min)	35
Hamiltonella	94 ^{0}C (4 Min)	94 ^{0}C (30 s)	52 ^{0}C (2 Min)	72 ^{0}C (2 Min)	35
Wolbachia	94 ^{0}C (4 Min)	94 ^{0}C (30 s)	55 ^{0}C (2 Min)	72 ^{0}C (2 Min)	35
Arsenophonus	94 ^{0}C (4 Min)	94 ^{0}C (30 s)	56 ^{0}C (2 Min)	72 ^{0}C (2 Min)	35
Cardinium	94 ^{0}C (4 Min)	94 ^{0}C (30 s)	52 ^{0}C (2 Min)	72 ^{0}C (2 Min)	35
Rickettsia	94 ^{0}C (4 Min)	94 ^{0}C (30 s)	58 ^{0}C (2 Min)	72 ^{0}C (2 Min)	35
Fritschea	94 ^{0}C (4 Min)	94 ^{0}C (30 s)	60 ^{0}C (2 Min)	72 ^{0}C (2 Min)	35
B. tabaci mtCOI	94 ^{0}C (1 Min)	94 ^{0}C (1 Min)	55 ^{0}C (1 Min)	72 ^{0}C (1 Min)	35

The products (5 µl) were visualized in 1.0% agarose gel containing ethidium bromide under UV illumination after a migration of 45 minute at 80 V. With the anticipated band size on the gels, the rest product (20 µl) was used for sequencing (Table 1). The obtained sequences were equated to the available sequences in the record using BLAST algorithm in NCBI.

2.5 Data Analysis

The variances in relative number of symbionts were investigated using one-way Analysis of Variance (ANOVA). Statistical evaluation was performed with SPSS version 16.0.

3. Results

The sequences of mtCOI *B. tabaci* from brinjal and tomato were analyzed and these revealed that the studied population goes to the Asia II 1 genetic group (Figure 1).

The results revealed the occurrence of seven known endosymbionts, *viz. Portiera, Cardinium, Rickettsia*, *Wolbachia*, *Arsenophonus*, *Fritschea* and *Hamiltonella*

infecting *B. tabaci* in the two hosts *viz.* brinjal and tomato. All the scanned individuals indicated the presence of primary endosymbiont, *Portiera*, which validates the high class of DNA extractions and Figure 2 shows the distribution frequency of endosymbionts. Apart from the *Fritschea* and *Hamiltonella,* individuals were found to be infected with S-endosymbionts.

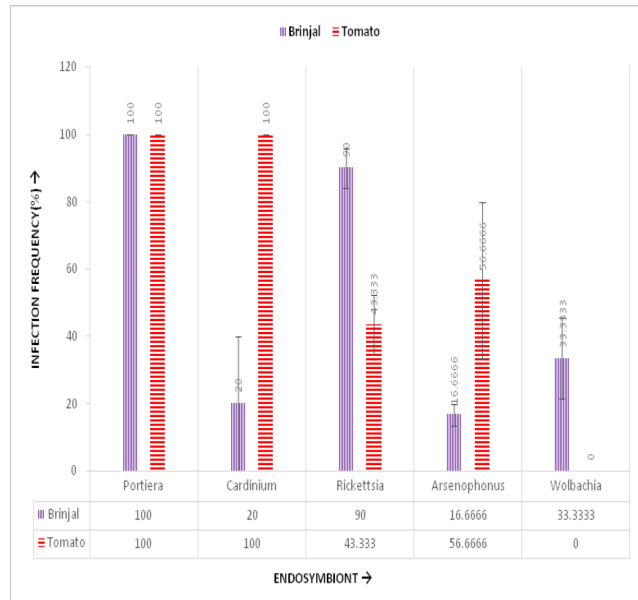

Figure 1. Distribution frequency of endosymbionts in solanaceous hosts of *Bemisia tabaci.*

In brinjal and tomato presence of the primary endosymbiont *i.e. Portiera aleyrodidarum* was 100%, while diversity was observed as regards the distribution of S-endosymbionts; percentage of *Wolbachia* was 33.34% in brinjal whereas it was totally absent in tomato; in case of *Rickettsia, Arsenophonus* and *Cardinium* the distribution frequency was 90% for brinjal and 43.34% in tomato, 16.67% in brinjal and 56.67% in tomato and 20% in brinjal and 100% in tomato, respectively.

Single factor ANOVA indicated that the infection of *Cardinium*, *Rickettsia* and *Wolbachia* vary significantly in the two hosts ($p = 0.016$, 0.011 and 0.050 respectively), whereas no significant variation was observed as regards *Arsenophonus* ($p = 0.165$).

In the Asia II 1 genetic group on the solanaceous hosts, distribution frequency varied significantly with the presence of *Cardinium* and *Arsenophonus* being higher in individuals from tomato as compared to brinjal, whereas individuals from brinjal have a higher level of *Rickettsia*. The presence of *Wolbachia* was about 33.34% in individuals obtained from brinjal but were entirely absent in the tomato population. These outcomes are in covenant with the prior studies[30–32], showing that within Q genetic group, most Q 1 individual's contains *Hamiltonella* and occasionally low frequencies of *Cardinium* and *Wolbachia*, however Q 3 individuals harbor frequently *Arsenophonus* with a high level of co-infection with *Rickettsia*.

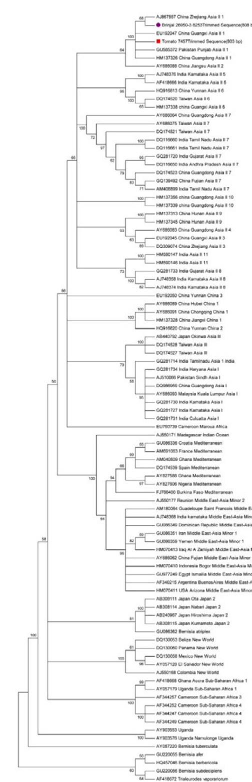

Figure 2. Showing the phylogenetic status of the *Bemisia tabaci* collected from solanaceous host plants.

4. Discussion

Arthropods harbors endosymbiotic bacteria producing a diversity of symbiotic links ranging from mutualism to parasitism[33,34]. The mutualistic symbiotic bacteria play an important role by providing vital nutrients which are deficient in arthropods feeding on imbalanced diets like plant sap[8]. In addition to providing nutrients, bacterial endosymbionts been recorded to have a variety of consequence on their hosts, *viz.* improved resistance to parasites[35], increasing the range of temperature tolerance[36] and also possibly have a role in the sexual selection of its insect hosts[37].

Bacterial endosymbionts are important for the existence, spread and development of the *B. tabaci*[13]. Bacterial diversity in *B. tabaci* has been studied by many workers from different pert of the world[30,31,38,39] but from India very few reports are available[40,41]. Thus, the present study was undertaken to study the bacterial endosymbiont diversity in *B. tabaci* nourishing on two solanaceous host plants, *viz*. brinjal and tomato.

During the present study, *B. tabaci* individuals collected from both the host plants was identified as belonging to Asia II 1 genetic group. A scan for identifying the symbionts diversity in brinjal and tomato host plants revealed a variation in the frequency distribution of the various S-endosymbionts within the same genetic group of *B. tabaci*. Thus this study confirms the association between the endosymbiotic bacterial groups and the genetic groups of *B. tabaci* and reach agreement with earlier works[30–32]. A variation was observed in the distribution of the S-endosymbionts in the hosts studied with 100% presence of P-endosymbiont *Portiera* in all the analyzed samples. The distribution frequency of secondary endosymbionts varied within the same genetic group within the Asia II 1 genetic group observed on the solanaceous host plants, distribution frequency varied significantly, the presence of *Cardinium* and *Arsenophonus* being higher in individuals from tomato compared to those from brinjal, whereas individuals from brinjal had a higher level of *Rickettsia*. *Wolbachia* was present only in about 33.34% in individuals feeding on brinjal but as entirely absent from the population on tomato. These outcomes are in covenant with the earlier studies[30–32], which indicated that within Q genetic group, most Q 1 individual's contains *Hamiltonella* and occasionally low incidences of *Wolbachia* and *Cardinium*, whereas Q 3 individuals harbour frequently *Arsenophonus* with a high level of co-infection with *Rickettsia*. In other words, there is a variation in the distribution of S-endosymbionts with the same genetic group of *B. tabaci*. Further the incidence of secondary symbionts diverse significantly between the different host plant within the same genetic group. A significant difference in the levels of *Cardinium*, *Rickettsia* and *Wolbachia* were observed in individuals from two hosts whereas for *Arsenophonus* it was not significant. This is the first study towards mapping of the endosymbiont diversity associated with hosts preference of *B. tabaci* in India. The results indicate a need for advanced studies on the host wise frequency distribution of S-endosymbionts and its relation with various genetic groups of *B. tabaci* and also their role if any in the polyphagus nature of this insect pest.

5. Acknowledgements

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6. Disclosure

The authors have no conflicts of interest, including specific economic interests and associations and affiliations relevant to the subject of this manuscript.

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