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Elimination of *Arsenophonus* and decrease in the bacterial symbionts diversity by antibiotic treatment leads to increase in fitness of whitefly, *Bemisia tabaci*

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ABSTRACT

Bemisia tabaci is an invasive agricultural pest with more than 24 genetic groups harboring different bacterial endosymbionts categorized into obligatory and facultative endosymbionts. *Arsenophonus* is one of the facultative endosymbionts prevalent in *B. tabaci* of Indian sub-continent. Not much is known about the functional role of this endosymbiont in its host. Some studies have revealed its involvement in virus transmission by *B. tabaci*, but how it effects the biology of *B. tabaci* is unknown. In this study, tetracycline was used to eliminate *Arsenophonus* from *B. tabaci* to study its effects with regard to development and other fitness parameters. Bacteria specific 16S Polymerase chain reaction (PCR) was used to ascertain *Arsenophonus* absence with differential effects on other secondary endosymbionts present in *B. tabaci*. Our results revealed that *Arsenophonus* negative (A^-) whiteflies had more fecundity, increased juvenile developmental time, increased nymphal survival and increased adult life span as compared to control (A^+) whiteflies. Thus, our results demonstrate that A^+ whiteflies have lesser fitness as compared to A^- whiteflies. These observations give a new insight about the probable role of *Arsenophonus* in *B. tabaci*, that need to be explored further.

1. Introduction

Bemisia tabaci (Gennadius), (Homoptera: Aleyrodidae) is a worldwide pest of agricultural crops and ornamental plants (Ahmed et al., 2009). They feed exclusively on phloem sap that is rich in carbohydrates and lacking in essential amino acids. The absence of essential amino acids in diet is suggested to be ameliorated by endosymbionts in these insects (Buchner, 1965; Su et al., 2013; Solan and Moran, 2012). Endosymbionts in insects have been categorized into two types- primary endosymbionts and secondary endosymbionts. Primary or obligatory endosymbionts found in bacteriocytes (Moran and Telang, 1998) are vertically transmitted and are thought to synthesize essential non-dietary metabolites (Clark et al., 2010; Douglas, 2009). The secondary or facultative endosymbionts are also vertically transmitted from mother to offspring and can also be transmitted horizontally through direct or indirect contact with other infected individuals (Clark et al., 2010; Feldhaar, 2011; Baumann et al., 2006). Although the role of different facultative bacterial endosymbionts in *B. tabaci* is largely unknown, there are evidences which point toward their contribution to the biological differences observed among different genetic groups (Gueguen et al., 2010) with some having competence to act as reproductive manipulators of their host (Moran et al., 2008; Feldhaar, 2011). In fact, different combinations of secondary endosymbionts have been associated with different genetic groups of whiteflies (Biotypes- Q and B) (Chiel et al., 2007; Costa et al., 1995). Though, the biological contribution of bacterial endosymbiont community to their host, *B. tabaci* has been well explored (Xue et al., 2012; Himler et al., 2011; Su et al., 2013; Fang et al., 2014), the importance to the host of specific endosymbionts has not been established.

Portiera aleyrodidarum is the only primary endosymbiont of whitefly (Baumann, 2005), while secondary endosymbionts include a range of bacteria like *Wolbachia* (Rickettsiales); (Zchori-fein and Brown, 2002), *Arsenophonus* (Enterobacteriales); (Thao

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and Baumann, 2004), *Cardinium* (Bacteroidetes); (Weeks et al., 2003), *Rickettsia* (Rickettsiales); (Gottlieb et al., 2006), *Hamiltonella* (Enterobacteriales); (Zchori-fein and Brown, 2002) and *Fritschea* (Chlamydiales); (Everett et al., 2005). Different genetic groups of *B. tabaci* have been known to carry different secondary endosymbionts. Endosymbionts have a variety of effects on their hosts such as increasing tolerance to heat stress (Montllor et al., 2002), increasing resistance to parasitic wasps (Oliver et al., 2005) and causing host plant specialization (Tsuchida et al., 2004). In fact, several secondary endosymbionts appear to affect the capability of the host insect to be a pest. *Hamiltonella* and *Arsenophonus* have been described to have significant contribution in virus transmission in plants (Gottlieb et al., 2010; Rana et al., 2012). Role of *Rickettsia* in heat tolerance and increased susceptibility to some insecticides like imidacloprid, thiamethoxam and pyriproxyfen is well documented (Brumin et al., 2011; Kontsedalov et al., 2008). It was also reported to play an important role in increasing fecundity, survival to adulthood and reduction in development time (Himler et al., 2011).

However, the full range of functions of different endosymbionts have not been extensively studied and the relationship between *B. tabaci* and its endosymbionts is partly understood because of the technical difficulty in culturing most of the endosymbionts *in vitro*. The role of endosymbionts in whitefly may be determined if they could be removed *in vivo* by using specific antibiotics. Different studies have assessed the effect of different antibiotics on different bacterial endosymbionts. Ruan et al. (2006), studied the effect of three antibiotics – tetracycline, ampicillin trihydrate and rifampicin on the fitness of different genetic groups of *B. tabaci*. Their results confirmed the removal of three secondary endosymbionts *Arsenophonus*, *Hamiltonella* and *Wolbachia*. Ahmed et al. (2010), showed *in vivo* sensitivity of endosymbionts in three genetic groups (biotypes- B, Q and Cv) to ampicillin, rifampicin and tetracycline, where *P. aleyrodidarum* was found to be unaffected by these treatments while the antibiotics had substantial effect on secondary endosymbionts. However, the 3 genetic groups showed different responses to different antibiotics. Xue et al. (2012), showed elimination of *Wolbachia* in *B. tabaci* Mediterranean with rifampicin that inturn affected whitefly development and reproduction. Thus, these studies confirmed the elimination of secondary endosymbionts by use of specific antibiotics which further helped in studying the role of bacterial endosymbionts. Keeping in view the literature, we used this approach of removing secondary endosymbiont *Arsenophonus* in *B. tabaci* and studied the functional role of this bacterium by comparing the life history traits of treated (A^-) and untreated (A^+) whiteflies.

2. Material and methods

2.1. Whitefly culture

B. tabaci was collected from cotton plant from the fields of Indian Agricultural Research Institute, Delhi and then maintained in insect proof climate control chambers. The population was reared on cotton at 27 ± 2 °C, photoperiod of 14:10 h (L:D) and 60–70% relative humidity. The genetic group was identified using mitochondrial cytochrome oxidize 1 (mtCO1) gene markers (Singh et al., 2012).

2.2. DNA extraction from B. tabaci for detection of endosymbionts

Individual whiteflies were used for DNA isolation. The whiteflies were washed in 200 μL of autoclaved water and then homogenized with the help of hand held homogenizer (Pellet pestles cordless motor, SIGMA–ALDRICH, Z359971-1EA) in 14 μL of lysis buffer (100 mM Tris–Cl pH 8.0, 1% SDS, 100 mM NaCl and 100 mM EDTA pH-8, 1%). 2 μL of Proteinase K (0.28 μg/μL; SIGMA–ALDRICH Catalog No. 39450-01-6) was added in the homogenized mixture and incubated at 65 °C for 45 min. After incubation 20 μL of pre-chilled 5 M potassium acetate and 8 μL of 6 M lithium chloride was added in the incubated homogenate and kept in ice for 15 min. The mixture was centrifuged at 10,000 rpm for 15 min. After centrifugation, the supernatant was taken and 0.6 volume of isopropanol was added. The supernatant and isopropanol mixture was again centrifuged at 10,000 rpm for 15 min. The pellet obtained after centrifugation was washed in 70% ethanol. After ethanol wash, the pellet was air dried and dissolved in elution buffer (10 mM Tris–Cl, pH 8.0) followed by RNase (0.1 μg/μL) treatment for 45 min at 37 °C. The DNA was then checked on 0.5% agarose gel containing ethidium bromide (0.5 μg/mL) by running it at 110 volts for 15 min and then observed using UV transilluminator (FOTODYNE incorporated, USA).

2.3. Diagnostic PCR for detection of bacterial endosymbionts in B. tabaci population

B. tabaci population was taken from insect proof climate control chambers at IARI, Pusa, Delhi. Fifty whiteflies were collected randomly for the experiment and diagnosed for the presence of different bacterial endosymbionts- *Portiera*, *Wolbachia*, *Rickettsia*, *Arsenophonus* and *Cardinium*. Specific bacterial primers were used for amplification of 16S rRNA bacterial gene (Table 1). For each bacterial endosymbiont, PCR mix consisting of dNTPs (2.5 mM), 1× buffer (2.5 μL), Taq polymerase (1 U), Forward and Reverse primers (7.5 pmol each), DNA template (25–30 ng) was prepared and the final volume was raised up to 25 μL with autoclaved mili-Q (MQ) water (Millipore corporation water purifying system).

PCR conditions involved denaturation at 94 °C for 30 s and annealing at different temperatures specific for each bacterial endosymbiont (*Portiera* 58 °C, *Wolbachia* 52 °C, *Arsenophonus* 55 °C, *Rickettsia* 55 °C, *Cardinium* 50 °C) for 30 s. Extension was carried out at 72 °C for 40 s with the final extension for 5 min at 72 °C. Forty-five number of cycles were fixed for each bacterial endosymbiont detection.

The plasmids containing 16S rRNA gene of different bacterial endosymbiont were used as positive controls while the reaction

Table 1
Primers and PCR cycling conditions for the identification of bacterial endosymbionts associated with *B. tabaci*.

Endosymbiont	Primer sequence	PCR cycle	Annealing temp	Product size	References
Portiera	F-5′TGCAAGTCGCGGCATCAT3′ R-5′CCGCCTTCTGCGTTGGCAACT3′	45	58 °C	1000 bp	Singh (2013)
Wolbachia	F-5′CGGGGGAAAATTTATTGCT3′ R-5′AGCTGTAATACAGAAAGGAAA3′	45	52 °C	650 bp	Singh et al. (2013)
Rickettsia	F-5′GCTCAGAACGAACGCTGG3′ R-5′GAAGGAAAGCATCTCTGC3′	45	55 °C	800 bp	Gottlieb et al. (2006)
Arsenophonus	F-5′CGTTTGATGAATTCATAGTCAAA3′ R-5′GGTCCTCCAGTTAGTGTTACCCAAC3′	45	52 °C	630 bp	Singh et al. (2013)
Cardinium	F-5′GCGGTGTAAAATGAGCTTG3′ R-5′ACCTCTTCTTTAACTCAAGCCT3′	45	50 °C	440 bp	Weeks et al. (2003)