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# Secondary endosymbionts distribution in Bangladesh whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae)

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**Abstract**: The sweetpotato whitefly, *Bemisia tabaci*, harbors of all most six secondary endosymbionts such as *Arsenophonus*, *Cardinium, Fritschea*, *Hamiltonella, Rickettsia* and *Wolbachia*. These bacteria play important roles to insect physiology. *Bemisia tabaci* is a species complex composed of more than 24 biotypes, which may diverge from each other both hereditarily and morphologically. The presence of secondary endosymbiont into the whiteflies was varied from biotype to biotype and strain to strain. Secondary endosymbionts infection occurrence in *B. tabaci* from different host-plants at different places in Bangladesh was determined by Polymerase Chain Reaction (PCR), in order to test for correlation between bacterial composition to biotype, host-plants and TYLCV transmission. *Arsenophonus, Cardinium, Hamiltonella* and *Wolbachia* were detected in all of the populations of the whiteflies from different places in Bangladesh that were collected from different host-plants, at the same time *Fritschea* and *Rickettsia* did not found in any whitefly populations of Bangladesh. No significant differences in secondary endosymbionts were found among host-plants within the whitefly population in Bangladesh. Secondary endosymbionts recommends a potential contribution of these bacteria to host-plant traits such as TYLCV transmission, insecticide resistance and host ranges.
**Key words:** *Arsenophonus*, *Bemisia tabaci*, *Cardinium*, *Hamiltonella, Wolbachia*

## Introduction

The sweetpotato whitefly Bemisia tabaci (Gennadius) (Homoptera: Aleyrodidae) known as cotton or tobacco whitefly, is a strange species of multifarious that includes of more than 24 different biotypes throughout tropical and sub-tropical regions around the World (Ahmed et al., 2009). B and Q biotypes are the most destructive among the all. As like as B, Q biotype has the capability to increase high population densities in a short period of time (Moya et al., 2001) along with high level of resistance to insecticides (Horowitz et al., 2005). Rapid development of chemical resistance, asymmetric mating displacement, and/or the presence of specially Wolbachia secondary endosymbionts might be important factors in the aggressive spread of the B biotype (Liu et al., 2007). Endosymbionts of insects are usually categorized into two types: primary endosymbionts and secondary endosymbionts. Primary endosymbionts are morphologically similar to each other and are harbored in bacteriocytes (Baumann et al., 2000), have been provisionally designated as Candidatus 'Portiera aleyrodidarum' (Thao and Baumann, 2004). Portiera aleyrodidarum, the obligatory primary endosymbiont of whiteflies, provides its host with essential nutrients like amino acids which are essential for host growth and reproduction and has long co-evolutionary history with all members of the Aleyrodinae subfamily (Thao and Baumann, 2004b). Secondary endosymbionts are morphologically different and are not restricted to bacteriocytes present in almost all types of cells of the host insect (Baumann et al., 2000). The property of six additional facultative secondary endosymbionts in the body of vector insect has yet to be determined, and have also been identified (Zchori-Fein and Brown, 2002; Nirgianaki et al., 2003). Four of them (Wolbachia, Cardinium, Rickettsia and Arsenophonus) are known to manipulate host reproduction in a wide range of insect species (Werren et al., 2008). One (Hamiltonella defensa) induces parasitoid resistance in the pea aphid (Oliver et al., 2002), and one (Fritschea bemisiae) has unknown effect and has so far only been reported in B. tabaci (Thao et al., 2003). Although B. tabaci hosts all these bacteria with indications for nonrandom distribution among biotypes (Chiel et al., 2007), the diversity of the whole secondary endosymbiotic community and its variation at different geographic and phylogenetic scales remains unknown. In addition, B. tabaci can harbors several secondary endosymbionts, such as Candidatus 'Hamiltonella defense' (Enterobacteriaceae); Wolbachia, Arsenophonus, Cardinium (Bacteroidetes); and Fritschea bemisiae (Simkaniaceae) (Everett et al., 2005). Different biotypes of B. tabaci can harbor different secondary endosymbionts (Nirgianaki et al. 2003); Wolbachia and Arsenophonus were found in both the Q and ZHJ-1 biotypes but not in the B biotype (Ruan and Liu, 2005; Chiel et al., 2007). Wolbachia has been reported in all major orders of insects (Li et al., 2007) and the infection rate of species level ranges from about 20% to more than 50% (Tagami and Miura, 2004; Kyei-poku et al., 2005). Taking the whole endosymbionts community into account is especially important to understand B. tabaci complex, which has one of the highest number of endosymbiotic elements, with seven different vertically transmitted bacteria reported so far (Zchori-Fein and Brown, 2002). Here, we analyzed the diversity, prevalence and distribution of all known endosymbionts in B. tabaci populations at the different places in Bangladesh, focusing on the correlate the composition of the endosymbiotic community in B. tabaci populations. In this study, the genetic differences among the B. tabaci populations from different host-plants in Bangladesh were investigated, for determination of secondary endosymbiont in *B. tabaci.*

## Materials and Methods

**Whiteflies collection:** Samples of Adult *B. tabaci* were collected from different places on different host-plants (bean, ridge gourd, pepper, tomato and eggplant) from Bangladesh in 2010 and were immediately preserved in 99% ethanol (absolute alcohol) and stored at -20°C.

**DNA extraction:** Total genomic DNA was extracted from individual *B. tabaci* according to protocol supplied by Invitrogen Purelink Genomic DNA mini kit. After

removing the sample from ethanol had been washed with double-distilled water to remove alcohol. Individual whiteflies were homogenized in 180 µl genomic digestion buffer using a 1.5 ml microcentrifuge tube and micropestle (homogenizer). Then added 200 µl genomic lysis/ binding buffer (1% SDS, 10 mM Tris-HCl, pH 8.0, 25 mM EDTA, 25 mM NaCl, Proteinase K 200 mg/ml) and after that immediately added 200 µl absolute ethanol. Subsequently added wash buffer into the genomic column and finally added 20 µl genomic elusion buffer (Invitrogen Purelink, Carisbad, CA, USA). Samples were centrifuged (12000 rpm for 1 minute) and incubated at RT (20°C) for 1 min. After that the supernatants/pellets were directly used for PCR amplification for detecting secondary endosymbionts or were stored at -20°C for later use. Total genomic DNA was extracted from each individual for further analysis (Dellaporta *et al.*, 1983).

**Primer design and PCR amplification:** The presence of secondary endosymbionts in the whitefly populations in Bangladesh was determined using the primers listed in Table 1. PCR reactions were performed in a 20 µl mixture containing 5 x SuperTaq PCR buffer (10 mM Tris-HCL, 40 mM KCl, 1.5 mM $MgCl_2$, pH 9.0), 2.5 mM dNTPs, 0.5 µM of each primer, 1 unit of SuperTaq DNA polymerase (SuperBio Co, Korea) and 1 µg of DNA as a template. The mixtures were amplified in a PTC-200 thermal cycler (MJ Research, Watertown, MA, USA) with a 3 min initial denaturation at 95°C, 35 cycles (30 sec at 94°C, 30 sec at 52~60°C, 30 sec at 72°C), and finally by a 10 min extension at 72°C. Annealing temperatures of each gene were listed in Table 2.

**Table 1.** Primer list for secondary endosymbiont detection

| Secondary Endosymbiont | Targeted Gene | Primer Direction | Primer Sequence (5' to 3') | Size(bp) | Reference | Annealing Temp.(cycle) |
|---|---|---|---|---|---|---|
| Arsenophonus | 23S rDNA | Forward<br>Reverse | CGTTTGATGAATTCATAGTCAAA<br>GGTCCTCCAGTTAGTGTTACCCAAC | ~600 | Thao and Baumann, 2004 | 60°C (30) |
| Cardinium | 16s rDNA | Forward<br>Reverse | GCGGTGTAAAATGAGCGTG<br>ACCTMTTCTTAACTCAAGCC | ~400 | Weeks et al., 2003 | 58°C (35) |
| Fritschea | 23s rDNA | Forward<br>Reverse | GATGCCTTGGCATTGATAGGCGATGAAGGA<br>TGGCTCATCATGCAAAAGGCA | ~600 | Everett et al., 2005 | 60°C (30) |
| Hamiltonella | 16s rDNA | Forward<br>Reverse | TGAGTAAAGTCTGGAATCTGG<br>AGTTCAAGACCGCAACCTC | ~700 | Zchori-Fein & Brown, 2002 | 58°C (35) |
| Rickettsia | 16s rDNA | Forward<br>Reverse | GCTCAGAACGAACGCTATC<br>GAAGGAAAGCATCTCTGC | ~900 | Gottlieb et al., 2006 | 60°C (30) |
| Wolbachia | 16s rDNA | Forward<br>Reverse | CGGGGGAAAAATTTATTGCT<br>AGCTGTAATACAGAAAGTAAA | ~625 | Zhou et al., 1998; Heddi et al, 1999 | 55°C (35) |

**Table 2.** PCR reaction used to detect secondary endosymbiont

| Endosymbiont | Targeted Gene | Pre-denaturation | Denaturation | Cycling conditions | | |
|---|---|---|---|---|---|---|
| | | | | Annealing | Extension | Cycles |
| Arsenophonus | 23SrDNA | 95°C (5 min) | 95°C (30 sec) | 60°C (30 sec) | 72°C (45 sec) | 30 |
| Cardinium | 16SrDNA | 95°C (5 min) | 94°C (1 min) | 58°C (1 min) | 72°C (1 min) | 35 |
| Fritschea | 23SrDNA | 95°C (5 min) | 95°C (30 sec) | 60°C (30 sec) | 72°C (45 sec) | 30 |
| Hamiltonella | 16SrDNA | 95°C (5 min) | 94°C (1 min) | 58°C (1 min) | 72°C (1 min) | 35 |
| Rickettsia | 16SrDNA | 95°C (2 min) | 92°C (30 sec) | 60°C (30 sec) | 72°C (30 sec) | 30 |
| Wolbachia | 16SrDNA | 95°C (5 min) | 95°C (30 sec) | 55°C (30 sec) | 72°C (1 min) | 35 |

**Gel-electrophoresis:** Amplified PCR products (5 µl) were electrophoresis using 1.0% agarose gels with 1X TAE at 100 V for 30 mins with 100bp ladder DNA marker and the gels were then stained by 10 µl Ethidium Bromide for 20 mins. When bands with the expected size were visible on the gels, then the rest of 15 µl of PCR products were used for sequencing. (The PCR products were visualized on a 1.0% agarose gel containing ethidium bromide. Expected PCR products were excised from the gel and purified using the Wizard PCR preps DNA purification system (Promega, Madison, WI, USA) and sequenced either directly or by cloning into the pGEM-T easy plasmid vector (Promega, Madison, WI, USA).)

**Sequence analysis:** The sequences of PCR products were determined using the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, USA) and analyzed by 3730XL DNA Sequencer (Applied Biosystems, Foster City, USA). Databases were searched using the BLAST algorithm (Altschul *et al.*, 1997) in NCBI and sequences were aligned using the MUSCLE program (Edgar, 2004). Mitochondrial COI sequences of *B. tabaci* were analyzed using MrBayes 3.0 software. Bayesian software MrBayes 3.0 four metropolises coupled with Markov Chain Monte Carlo (MCMC) chains were run, stopping when the standard divergence of split frequencies was less than 0.01 (Ronquist and Huelsenbeck, 2003). All sequences were analyzed over 10 million generations and four were sampled every 100 generations and the first 25% burn-in (SUMP and SUMT) cycles were discarded prior to the construction of the consensus tree. Consensus trees were visualized with MEGA 4.0 (Tamura *et al.*, 2007).

## Results

Secondary endosymbiont were detected in different whiteflies from different places. Sweetpotato whitefly samples that were collected from Bangladesh are containing endosymbiotic bacteria. They were shown the

fragments of appropriate size in PCR amplification (Fig. 1, Table 1) to 16S-23S rDNA primer so that the indigenous biotype (Non B/Q) of *B. tabaci* samples of Bangladesh always harbored four endosymbionts *Arsenophonus, Cardinium, Hamiltonella* and *Wolbachia* commonly in its body among the six (Table 3).

**Table 3.** Profile of secondary endosymbiotic bacteria in indigenous whiteflies on different host plants of Bangladesh

| Species | Biotype | Host Plants | Locations | Endosymbiotic Bacteria | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | A | C | F | H | R | W |
| B. tabaci | Non-B/Q | Bean | Patuakhali, Bangladesh | + | + | - | - | - | + |
| B. tabaci | Non-B/Q | Bean | Barguna, Bangladesh | + | + | - | + | - | + |
| B. tabaci | Non-B/Q | Eggplant | Patuakhali, Bangladesh | + | + | - | + | - | + |
| B. tabaci | Non-B/Q | Eggplant | Barisal, Bangladesh | + | + | - | + | - | + |
| B. tabaci | Non-B/Q | Tomato | Patuakhali, Bangladesh | + | + | - | + | - | + |
| B. tabaci | Non-B/Q | Tomato | Bhola, Bangladesh | + | + | - | + | - | + |

(A: *Arsenophonus,* C: *Cardinium,* F: *Fritschea,* H: *Hamiltonella,* R: *Rickettsia,* W: *Wolbacchia*)

**Table 4.** Sequence of 16S-23S rDNA region of different secondary endosymbiotic bacteria in *B. tabaci* of Bangladesh

| Endosymbiotic bacteria | Sequence of 16-23S ribosomal DNA region of whitefly | Length (bp) |
|---|---|---|
| Arsenophonus (GenBank accession no.: JN018060) | CTCAGTACCCCGAGGAAAAGAAATCAACCGAGATTCCCCCAGTAGCGGCGAGCGAACGGGGAGCAGCCCAG AGTCAACATCAATATTTACCGCAGGAGAAGGGTCTGGAAAGGCCGGCAATAAAGGGTGATAGCCCCGTATCT GAAACGGTAAGTGTTGTGAACTCGAAGAGTAGGGCGGGACACGTGTTATCCTGTCTGAATATGGGGGGACCA TCCTCCAAGGCTAAATACTCCTGACTGACCGATAGTGAACCAGTACCGTGAGGGAAAGGCGAAAAGAACCCC GGCGAGGGGAGTGAAATAGAACCTAAAACCGTGTACGTACAAGCAGTGGGAGCACCCAAAAGGGTGTGACT GCGTACCTTTTGTATAATGGGTCAGCGACTTATATTCTGTAGCAAGGTTAACCGGATAGGGGAGCCGTAGGGA AACCGAGTCTTAACTGGGCGTTAAGTTGCAGGGTATAGACCCGAAACCCGGTGATC | 486 |
| Cardinium (GenBank accession no.: JN018062) | TGGTGAGGTAATGGCTCACCAAGGCTACGATGGGTAGGGGTTCTTAGTGGAAGGTCCCCCACACTGGCACTG AGATACGGGCCAGACTCCTACGGGAGGCAGCAGTAGGGAATATTGGTCAATGGGCGCAAGCCTGAACCAGC CATGCCGCGTGCAGGATGAAGGCTCTCTGAGTTGTAAACTGCTTTTGTACAGGAGCAAAAAAATCCCTGCGG GGGTTCTTGAGAGTACTGTAAGAATAAGCACCGGCTAATTCCGTGCCAGCAGCCGCGGTAATACGGGAGGTG CAAGCGTTATCCGGTTTTATTGGGTTTAAAGGGTGCGTAGGCGGCTTATTAAGTCAGTTGTGAAATCCTAGTG CTTAACGCTAGAACT | 378 |
| Hamiltonella (GenBank accession no.: JN018063) | CGGCAGCTAATACCGCATGAAGTCGTGAGACCAAAGTGGGGGACCTTCGGGCCTCACGCCGTCGGATGAGCC CAGATGAGATTAGCTGGTAGGTAGGGTAAAGGCTTACCTAGGCGACGATCTCTAGCGGGTCTGAGAGGATAG CCCGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGG CGAAAGCCTGATGCAGCCATGCCACGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGGAGG AAGCGATAAATGCCAATACCATTTATTTTTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCA GCCGCGGTAATACGGAGGGTGCGAGCGTTAATCGGAATAACTGGGCGTAAAGGGCATGTAGGCGGTGAGTTA AGTCAGATGTGAAATCCCCGAGCTCAACTTGGGAATGGCATTTGAAACTGGGTCGCTAGAGTTTTCTAGAGG GGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGATATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCC TGGAGAAAGACTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCA | 642 |
| Wolbachia (GenBank accession no.: JN018064) | ATTAGATTAGCTAGTTGGTGGAGTAATAGCCTACCAAGGCAATGATCTATAGCTGATCTGAGAGGATGATCA GCCACACTGGAACTGAGATACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCG AAAGCCTGATCCAGCTATGCCGCATGAGTGAAGAAGGCCTTTGGGTTGTAAAGCTCTTTTAGTGAAGAAGAT AATGACGGTACTCACAGAAGAAGTCCTGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGAGGGCTAGC GTTATTCGGAATTATTGGGCGTAAAGGGCGCGTAGGCTGATTAATAAGTTAAAAGTGAAATCCCGAGGCTTA ACCTTGGAATTGCTTTTAAAACTATTAATCTAGAGATTGAAAGAGGATAGAGGAATTCCTGATGTAGAGGTAA AATTCGTAAATATTAGGAGGAACACCAGTGGCGAAGGCGTCTATCTGGTTCAAATCTGACGCTGAGGCGCGA AGGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTATAAACGAT | 558 |

To determine endosymbiont infection of *B. tabaci*, the presence of 6 endosymbiotic bacteria in Bangladesh populations of *B. tabaci* from different host-plants were examined by PCR analysis of 16S or 23S rDNA sequences (Fig. 1). *Arsenophonus, Cardinium, Hamiltonella* and *Wolbachia* were detected in all the tested populations of Bangladesh indigenous biotypes. However, *Rickettsia* and *Fritschea* were not detected in any populations of Bangladesh.

**Endosymbiont distribution:** All the populations from different host plants examined in this study were infected with always four secondary endosymbionts (Table 3). Non-B/Q biotype was co-infected by *Arsenophonus, Cardinium, Hamiltonella* and *Wolbachia*. In contrast, *Fritschea* and *Rickettsia* were not found in the same host.

**Sequence analysis:** After amplification of 16S-23S rDNA region was cloned and sequenced (Table 4). The provided sequences shared 100% similarities with released sequences of same endosymbiotic bacteria (*Arsenophonus*, *Cardinium*, *Hamiltonella* and *Wolbachia*) in NCBI database (Table 4). Genetic distance among 12 species including Bangladesh *Arsenophonus*, *Cardinium*, *Hamiltonella* and *Wolbachia* in *B. tabaci* based on 16S-23S rDNA sequences calculated by Kimura-2-parameter model in MEGA 4.

**Phylogenetic analysis:** The Neibour-joining phylogenetic tree reconstruction based on sequence of four different endosymbiotic bacteria (*Arsenophonus*, *Cardinium*, *Hamiltonella*, and *Wolbachia*) from Myanmar and Korea were compared with *Arsenophonus*, *Cardinium*, *Hamiltonella*, and *Wolbachia* in Bangladeshi *Bemisia tabaci* respectively are shown in Fig.2. It revealed that the sequences of Bangladeshi *Arsenophonus*, *Cardinium*, *Hamiltonella*, and *Wolbachia* from *B. tabaci* were clustered with Myanmar and Koreans *Arsenophonus*, *Cardinium*, *Hamiltonella*, and *Wolbachia* respectively (Fig. 2).

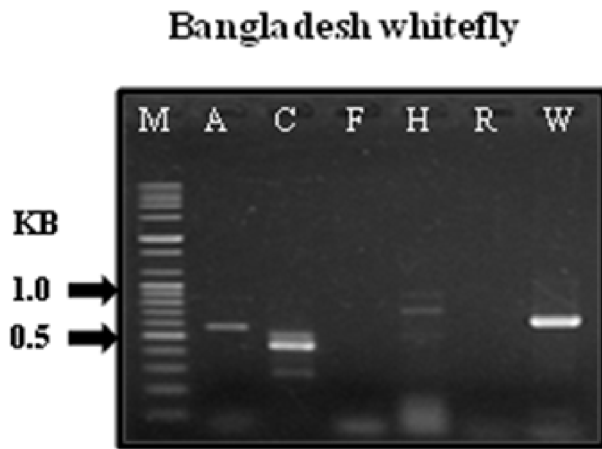


**Fig. 1.** 1% Agarose gel electrophoresis amplified by PCR product for secondary endosymbiont detection in indigenous whitefly of Bangladesh, M: DNA marker, A: *Arsenophonous*, C: *Cardinium*, F: *Fritschea*, H: *Hamiltonella*, R: *Rickettsia* and W: *Wolbachia*

## Discussion

The experiment demonstrates a clear association between certain secondary endosymbiont and *B. tabaci* populations in Bangladesh. The whitefly populations, those were collected from Bangladesh are indigenous biotype and harbours of *Arsenophonus*, *Cardinium*, *Hamiltonella* and *Wolbachia*. Previously, reported that all Israeli populations of the B biotype harbor *Hamiltonella*, whereas *Wolbachia* and *Arsenophonus* were found exclusively in the Q biotype (Chiel *et al.* 2007). Variability of symbiont combinations has been reported in the past for the A and B biotypes (Costa *et al.* 1995), although at that time the identity of the bacteria was not exposed. It thus appears that biotype-dependent or host plant-dependent differences of secondary endosymbionts composition can be of use in differentiating between *B. tabaci* biotypes (Chiel *et al.* 2007). Q biotype of *Bemisia tabaci* in Croatia harboured *Cardinium*, *Hamiltonella*, *Rickettsia* and *Wolbachia*, while *Arsenophonus* and *Fritschea* were not detected in any populations of Q biotype in Croatia (Skaljac *et al.*, 2010). Secondary endosymbionts are usually considered nonessential to their hosts, hence their presence between and within populations can be variable. *Hamiltonella*, for example, was previously reported from 40% of *B. tabaci* populations (Zchori-Fein and Brown, 2002) and from 0 – 46% of screened pea aphid populations. The incidence of *Rickettsia* in *A. pisum* also ranged from 1– 48% in various reports (Chen *et al.*, 2000; Darby *et al.*, 2001; Tsuchida *et al.*, 2002; Darby *et al.*, 2003; Haynes *et al.*, 2003; Ferrari *et al.*, 2004). Exceptionally, all 40 clones of the aphid *Uroleucon ambrosiae* collected throughout the USA were found to carry *Hamiltonella* (Sandstrom *et al.*, 2001), that supports the occurrence of *Hamiltonella* in *B. tabaci* of Bangladesh.

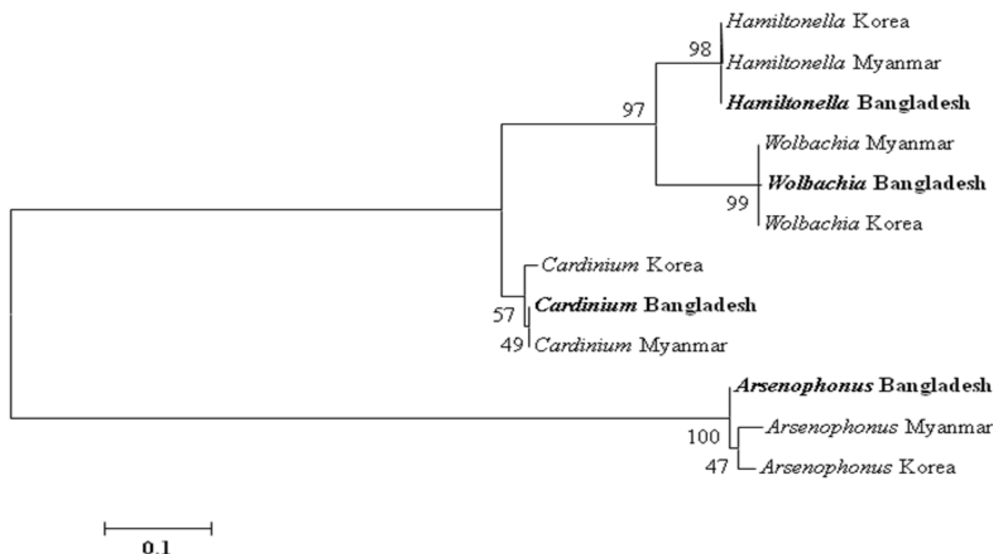


**Fig. 2.** Phylogenetic relationships of different secondary endosymbiont in *B. tabaci* populations based on a fragment (~378 bp) of the 16-23S ribosomal DNA sequences. Neibour-joining phylogenetic tree reconstructed using the same number of nucleotides of different endosymbionts in *B. tabaci* 16-23S rDNA sequences as a molecular marker according to the Bayesian method. The numbers placed at each node indicate the bootstrap support for values > 40. The horizontal branch length is drawn to scale, and the bar indicates 0.1 nt replacements per site.

Such a high incidence may indicate an obligatory or even mutualistic interaction between *Hamiltonella* and its host. There are many ways in which *Hamiltonella* may contribute to the whitefly. *Hamiltonella* might also be involved in plant physiological disorders that are exclusively caused by B biotype (e.g. squash silver-leafing) (Chiel *et al*., 2007). Both *Wolbachia* and *Arsenophonus* were found in the indigenous whitefly of Bangladesh. In our findings, *Wolbachia* can be found in all major insect orders in varying frequencies (Stouthamer *et al*., 1999; Werren & Windsor, 2000). In addition, it was reported that 11 out of 39 *B. tabaci* populations collected worldwide were infected with *Wolbachia*; of those, ten were 'non-silverleafing' biotypes (Nirgianaki *et al*., 2003). *Fritschea* and *Rickettsia* were not found in the whitefly of Bangladesh. In that study, 4 of 6 (*Arsenophonus, Cardinium, Hamiltonella* and *Wolbachia*) were found in the whitefly of Bangladesh.

## References


Ahmed, M.Z., Shatters, R.G., Ren, S.X., Jin, G.H., Mandour, N.S., Qiu, B.L., 2009. Genetic distinctions among the Mediterranean and Chinese populations of *Bemisia tabaci* Q biotype and their *Wolbachia* infection. *J. Appl. Entomol.* 133:733–741.

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.* 25:3389–3402.

Baumann, P., Moran, M.A., Baumann, L., 2000. Bacteriocyte-associated endosymbionts of insects. In Dworkin M (ed) The prokaryotes [Online.] New York: Springer, http://link.springer.de /link/ service/ books/10125.

Chiel, E., Gottlieb, Y., Zchori-Fein, E., Mozes-Daube, N., Katzir, N., Inbar, M., Ghanim, M., 2007. Biotype-dependent secondary symbionts communities in sympatric populations of *Bemisia tabaci*. *Bull. Entomol. Res.* 97:407–413.

Costa, H.S., Westcot, D.M., Ullman, D.E., Rosell, R., Brown, J.K., Johnson, M.W., 1995. Morphological variation in *Bemisia* endosymbionts. *Protoplasma* 189:194–202.

Darby, A.C., Birkle, L.M., Turner, S.L. & Douglas, A.E., 2001. An aphid-borne bacterium allied to the secondary symbionts of whitefly. *FEMS Microbiology Ecology* 36: 43–50.

Darby, A.C., Tosh, C.R., Walters, K.F.A. & Douglas, A.E., 2003. The significance of a facultative bacterium to natural populations of the pea aphid *Acyrthosiphon pisum*. *Ecological Entomology* 28: 145–150.

Dellaporta, S., Wood, J., and Hicks, J.B., 1983. A plant DNA minipreparation: version II. *Plant. Mol. Biol. Rept.* 1:19-21.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucl. Acids Res. (2004) 32 (5): 1792-1797.

Everett, K.D.E., Thao, M., Horn, M., Dyszynski, G.E., Baumann, P., 2005. Novel chlamydiae in whiteflies and scale insects:endosymbionts 'Candidatus Fritschea bemisiae' strain Falk and 'Candidatus Fritschea eriococci' strain Elm. *Int. J. Syst. Evol. Microbiol.* 55:1581–1587

Ferrari, J., Darby, A.C., Daniell, T.J., Godfray, H.C.J. & Douglas, A.E., 2004. Linking the bacterial community in pea aphids with host-plant use and natural enemy resistance. *Ecological Entomology* 29: 60–65.

Gottlieb, Y., Ghanim, M., Chiel, E., Gerling, D., Portnoy, V., Steinberg, S., Tzuri, G., Horowitz, A.R., Belausov, E., Mozes-Daube, N., Kontsedalov, S., Gershon, M., Gal, S., Katzir, N. & Zchori-Fein, E., 2006. Identification and localization of a *Rickettsia* sp in *Bemisia tabaci* (Homoptera: Aleyrodidae). *Applied and Environmental Microbiology* 72:3646–3652.

Haynes, S., Darby, A.C., Daniell, T.J., Webster, G., Van Veen, F.J.F., Godfray, H.C.J., Prosser, J.I. & Douglas, A.E. (2003). Diversity of bacteria associated with natural aphid populations. Applied and Environmental Microbiology 69, 7216–7223.

Heddi, A., Grenier, A.M., Khatchadourian, C., Charles, H., Nardon, P., 1999. Four intracellular genomes direct weevil biology: nuclear, mitochondrial, principal endosymbiont and *Wolbachia*. *Proc Natl Acad Sci USA*. 96:6814–6819.

Horowitz, A.R., Kontsedalov, S., Khasdan, V., Ishaaya, I., 2005. Biotypes B and Q of *Bemisia tabaci* and their relevance to neonicotinoid and pyriproxyfen resistance. *Arch. Insect Biochem. Physiol*. 58: 216–225.

Kyei-Poku, G.K., Colwell, D.D., Coghlin, P.C., Benkel, B. & Floate, K.D., 2005. On the ubiquity and phylogeny of *Wolbachia* in lice. *Molecular Ecology* 14: 285–294.

Li, Z.X., Lin, H.Z., Guo, X.P., 2007. Prevalence of *Wolbachia* infection in *Bemisia tabaci*. *Curr. Microbiol.* 54:467–471

Liu, S.S., De Barro, P.J., Xu, J., Luan, J.B., Zang, L.S., Ruan, Y.M., Wan, F.H., 2007. Asymmetric mating interactions drive widespread invasion and displacement in a whitefly. *Science* 318: 1769–1772.

Moya, A., Guirao, P., Cifuentes, D., Beitia, F., Cenis, J.L., 2001. Genetic diversity of Iberian populations of *Bemisia tabaci* (Hemiptera: Aleyrodidae) based on random amplified polymorphic DNA-polymerase chain reaction. *Molecular Ecology.* 10: 891-897.

Nirgianaki, A., Banks, G.K., Frohlich, D.R., Veneti, Z., Braig, H.R., Miller, T.A., Bedford, I.D., Markham, P.G., Savakis, C. & Bourtzis, K., 2003. *Wolbachia* infections of the whitefly *Bemisia tabaci*. *Current Microbiology.* 47: 93–101.

Oliver, K.M., Russell, J.A., Moran, A.N., Hunter, M.S., 2002. Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. Proceedings of *the National Academy of Sciences of the United States of America,* 100:1803–1807.

Ruan, Y.M., Liu, S.S., 2005. Detection and phylogenetic analysis of prokaryotic endosymbionts in *Bemisia tabaci*. *Acta. Entomol. Sin.* 48:859–865

Skaljac, M., Zanic, K., Ban, S. G., Kontsedalov, S. and Ghanim, M., 2010. Co-infection and localization of secondary symbionts in two whitefly species. *BMC Microbiology*, 10:142.

Stouthamer, R., Breeuwer, J.A.J. & Hurst, G.D.D., 1999. *Wolbachia pipientis*: Microbial manipulator of arthropod reproduction. *Annual Review of Microbiology* 53: 71–102.

Tagami, Y., Miura, K., 2004. Distribution and prevalence of *Wolbachia* in Japanese population of Lepidoptera. *Insect Mol. Biol*. 13 (4): 359–364.

Tamura, K., Dudley, J., Nei, M. & Kumar, S., 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24:1596-1599.

Thao, M., Baumann, P., 2004b. Evolutionary relationships of primary prokaryotic endosymbionts of whiteflies and their hosts. *Applied and Environmental Microbiology*, 70: 3401–3406.

Thao, M.L., Baumann, P., 2004. Evidence for multiple acquisition of Arsenophonus by whitefly species (Sternorrhyncha: Aleyrodidae). *Curr. Microbiol.* 48:140–144

Tsuchida, T., Koga, R., Shibao, H., Matsumoto, T. & Fukatsu, T., 2002. Diversity and geographic distribution of secondary endosymbiotic bacteria in natural populations of the pea aphid, Acyrthosiphon pisum. *Molecular Ecology* 11: 2123–2135.

Weeks, A.R., Velten, R. & Stouthamer, R., 2003. Incidence of a new sex-ratio-distorting endosymbiotic bacterium among

arthropods. Proceedings of *the Royal Society Series B* 270: 1857–1865.
Werren, J.H., Baldo, L., Clark, M.E., 2008. Wolbachia: master manipulators of invertebrate biology. *Nature Reviews Microbiology*, 6: 741–751.
Werren, J.H., Windsor, D.M., 2000. *Wolbachia* infection frequencies in insects: evidence of a global equilibrium? *Proc. R. Soc. Lond. Biol.* 267: 1277–1285.
Zchori-Fein, E., Brown, J.K., 2002. Diversity of prokaryotes associated with *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). *Annals of Entomological Society of America*, 95: 711–718.
Zhou, W., Rousset, F., O'Neill, S., 1998. Phylogeny and PCRbased classification of *Wolbachia* strains using wsp gene sequences. *Proc. R. Soc. Lond. Biol*. 265: 509–515.