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MOLECULAR DIVERGENCE OF SECONDARY ENDOSYMBIONT, *CARDINIUM* IN *BEMISIA TABACI* (GENNADIUS) AND ASSOCIATES

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Abstract

Whitefly, *Bemisia tabaci* (Hemiptera : Aleyrodidae) harbors numerous secondary endosymbionts, which are transmitted from mother to offspring by both horizontally and vertically, that have crucial role on host selection, biology, and evolution. Bacteria, *Cardinium* was identified in *B. tabaci* as well as in other whitefly population from many different countries by comparing 16S rDNA sequences. *Cardinium* were detected in all tested indigenous *B. tabaci* populations of Bangladesh, Myanmar, Nepal, and the Philippines as well as Q1 biotype of Korea. It was absent in B biotype of Korea and Q biotype of China. *Cardinium* was also detected in three out of five tested *Aleurodicus dispersus* population as well as in five out of seven *Trialeurodes vaporariorum*, whereas they were not detected in *Tetraleurodes acaciae* populatuion. In adition, *Cardinium* was detected in parasitoid *Encarsia formosa* attacking *B. tabaci*. Among the 19 whitefly populations from different countries, present studies identified four phylogenetic groups of *Cardinium*, thereby demonstrating the high diversity of this genus. *Cardinium* phylogeny suggests a correlation of geographical range with ecological variation at the species level.

Keywords: Molecular divergence, Intra-specific variation, *Aleurodicus dispersus*, Endosymbiont, *Bemisia tabaci*, *Cardinium*

Introduction

Endosymbiotic bacteria are harbored within the cells or tissues of many arthropods, including insects (Buchner, 1965; Brown *et al*., 1995; Frohlich *et al*., 1999). The symbiotic relationships between insects and endosymbionts drive evolutionary interactions, resulting in broad ranging activities from neutralism to ammensalism (Moran, 2007; Moya *et al*., 2008).

Endosymbionts can be categorized as either primary or secondary endosymbionts according to their physiological roles. The primary endosymbiont *Portiera aleyrodidarum* is harbored within bacteriocytes and supplements *B. tabaci* with

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essential amino acids for growth and development (Moran and Telang, 1998; Thao *et al*., 2000; Baumann, 2005). Although secondary endosymbionts are not necessary for host survival, they may play important roles in their host's physiology, ecology, and evolution (Zchori-Fein and Brown, 2002; Chiel *et al*., 2007). Until now, seven secondary endosymbionts have been identified in *B. tabaci*, namely *Arsenophonus*, *Cardinium*, *Fritschea*, *Hamiltonella*, *Orientia*, *Rickettsia*, and *Wolbachia* (Baumann, 2005; Gottlieb *et al*., 2006; Chiel *et al*., 2007; Bing *et al*., 2012).

Cardinium bacteria represent a clade within the Flexibacteraceae, which belongs to the class Sphingobacteria under the phylum Cytophaga-Flavobacterium-Bacteroides (CFB), a phylum that is unrelated to the α-Proteobacteria to which *Wolbachia* belongs. It was first seen in cell cultures established from the tick *Ixodes scapularis* (Kurtti *et al*., 1996). These unusual bacteria were observed in electron micrographs and in later studies their presence was confirmed by PCR and phylogenetic analysis. *Cardinium* are transovarially transmitted Gram-negative pleiomorphic rod-like bacteria about 1-2 micrometer long and approximately 0.5 micrometer wide. They have a distinctive parallel array of hollow filaments resembling microtubules that extend from the inner membrane into the cytoplasm (Bigliardi *et al*., 2006; Nakamura *et al*., 2009). *Cardinium* was originally named *Encarsia* bacterium (EB), (Zchori-Fein *et al*., 2001), and then, Cytophaga-like organisms (CLO), (Hunter *et al*., 2003), Cytophaga-Flavobacterium-Bacteroides (CFB) (Weeks and Breeuwer, 2003) and, finally, *Cardinium hertigii* (Zchori-Fein and Perlman, 2004; Zchori-Fein *et al*., 2004).

All bacteria possess the ability to manipulate the physiological characteristics of their hosts. Specifically, *Wolbachia*, *Arsenophonus*, *Cardinium*, and *Rickettsia* can manipulate host reproduction (Duron *et al*., 2008; Werren *et al*., 2008), *Hamiltonella* can induce virus resistance in pea aphid (Oliver *et al*., 2002). *Rickettsia* increases thermotolerance in *B. tabaci* (Brumin *et al*., 2011). However, in most cases, the presence of endosymbionts is indicated exclusively by polymerase chain reaction (PCR) using DNA template consequent from whole arthropods. Although no direct confirmation of symbiosis can be attained, a positive result strongly represents the symbiotic partners in question. The objectives of this study were to determine the geographic distribution of *Cardinium* infection in *B. tabaci* and other whiteflies as well as the relationship between *Cardinium* infection and evolutionary linkage in whiteflies.

Materials and Method

Collection of whitefly samples

Adult individuals of *B. tabaci* and other whiteflies were collected from various host plants, such as tomato, pepper, ridge gourd, bean, okra, eggplant, and guava grown in Bangladesh, Myanmar, Nepal, China, Philippines and Korea (Tables 2 and 3). In Bangladesh, we collected whiteflies from bean, okra, and eggplant as of the southern, northern and eastern parts of the country during 2011-2012, immediately preserved samples in 99% ethanol, and stored them at -20^{0}C for further molecular analysis. Adults of *B. tabaci* B and Q biotypes were collected from cucumber, sweet melon, and tomato plants which grown throughout Republic of Korea in 2010 in order to compare the morphologies and genetic sequences of foreign whiteflies. This research work has been performed from January 2011 to June 2012 at the insect molecular physiology lab in the Republic of Korea.

Identification of whiteflies, endosymbionts, and TYLCV

Different genotypic group of *B. tabaci* was determined by amplification the gene of mtCOI region causing specific fragments from extracted genomic DNA samples (Khasdan *et al*., 2005). The presence of *Cardinium* in whiteflies was determined using specific primer sets for *Cardinium* by amplification of 16S rDNA gene fragments as described by Chiel *et al*., 2007. The occurrence of *Tomato yellow leaf curl virus* (TYLCV) on farmhouses of various horticultural crops was surveyed in different geographical regions. Acquisition of this virus by *B. tabaci* was determined using a TYLCV-specific primer set that can amplify conserved intergenic sequences (*Lee et al*., 2010). Specific primer sets for biotypes, endosymbionts, and TYLCV are listed in Table 1. Polymerase chain reaction (PCR) reactions for *Cardinium* were performed in a 20 µl mixture containing 5× 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 DNA of whitefly as a template. The mixtures were amplified using a PTC-200 thermal cycler (MJ Research, Watertown, MA, USA) with 5 min of initial denaturation at 95°C, 35 cycles of annealing (1 min at 94°C, 1 min at 58°C, 1 min at 72°C), and 10 min of extension at 72°C. 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 pGEM-T easy plasmid vector (Promega, Madison, WI, USA).

Table 1. Nucleotide sequences of primer sets for identification of *B. tabaci* and its biotypes as well as detection of *Cardinium* and TYLCV.

Obs.	Targeted gene	Primer Sequence (5' to 3')	Size (bp)	References	Anneal. Temp.
B. tabaci	mtCOI	F-TTGATTTTTTGGTCATCCAGAAGT R-TCCAATGCACTAATCTGCCATATTA	~860	Simon *et al.*, 1994	52°C
Biotype	16S rRNA	F-CCGGTTTGAACTCAGATCATGT R-CGCCTGTTTAACAAAAACAT	~520	Simon *et al.*, 1994	55°C
Cardinium	16S rDNA	F-GCGGTGTAAAATGAGCGTG R-ACCTMTTCTTAACTCAAGCC	~400	Weeks *et al.*, 2003	58°C
TYLCV	Coat Protein	F-TGGGGATTCACAAATGTTTTCT R-CTGAACTTCGACAGCCCAT	~1000	Shatters *et al.*, 2009	50°C

DNA sequence analysis

Sequences of the PCR products were determined using a Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, USA) and analyzed using a 3730XL DNA Sequencer (Applied Biosystems, Foster City, USA). Databases were searched using the BLAST algorithm (Altschul *et al*., 1997; Schäffer *et al*., 2001) in NCBI, and sequences were aligned using the MUltiple Sequence Comparison by Log-Expectation (MUSCLE) program (Edgar, 2004). The16S ribosomal DNA sequences of *Cardinium* were analyzed using Bayesian MrBayes 3.0 software. Four Metropolises-coupled Markov Chain Monte Carlo (MCMC) chains were run until standard divergence of the split frequencies become lower than 0.01 (Ronquist and Huelsenbeck, 2003). All sequences were analyzed over 10 million generations, and four sequences were sampled every 100 generations. The first 25% of burn-in (SUMP and SUMT) cycles were discarded prior to the construction of consensus tree, which were visualized by MEGA 4.0 (Tamura *et al*., 2007).

Results

Detection and identification of *Cardinium* in *B. tabaci* populations

To launch the intimate association between *B. tabaci* and infection of its harbored secondary endosymbionts in different biotypes, the presence of *Cardinium* in indigenous whitefly population from Bangladesh, Myanmar, Nepal, and Q biotype of China were examined by PCR amplification of 16S rDNA gene as well as sequences analysis (Table 2). *Cardinium* was detected in all tested whitefly populations regardless of location, but not in B biotype of Korea and Q biotype of China (Table 2).

Cardinium detection in other whiteflies

We collected many other genera of whiteflies from Korea, Bangladesh, Myanmar, Nepal, New Zealand, Japan, and the Philippines. We found that infection of *Cardinium* in spiraling whitefly (*Aleurodicus dispersus*) and greenhouse whitefly (*Trialeurodes vaporariorum*) was very rare (Table 3). On the other hand, *Cardinium* infection was apparently absent in examined acacia whitefly (*Tetraleurodes acaciae*) population (Table 3).

Table 2. Profile of secondary endosymbiotic bacteria in different biotypes of *B. tabaci* on different host plants.

Biotype	Host Plants	Locations	TYLCV	*Cardinium*
B	Cucumber	Goyang, Korea	-	-
Q1	Various	Various places, Korea	+/-	+
Q	Tomato	Qingdao, China	-	-
BW1	Bean	Patuakhali, Bangladesh	-	+
BW1	Bean	Patuakhali, Bangladesh	+	+
BW1	Eggplant	Patuakhali, Bangladesh	-	+
BW1	Eggplant	Patuakhali, Bangladesh	-	+
BW2	Eggplant	Kurigram, Bangladesh	+	+
BW2	Eggplant	Kurigram, Bangladesh	-	+
BW2	Okra	Kurigram, Bangladesh	-	+
Indigenous	Eggplant	Kyuktan, Myanmar	+	+
Indigenous	Ridge gourd	Kyuktan, Myanmar	+	+
Indigenous	Ridge gourd	Magway, Myanmar	-	+
Indigenous	Eggplant	Yangon, Myanmar	-	+
Indigenous	Marigold	Kathmandu, Nepal	-	+
Indigenous	Chili (Pepper)	Kathmandu, Nepal	-	+
Indigenous	Cucumber	Kathmandu, Nepal	-	+
Indigenous	Brinjal	Kathmandu, Nepal	+	+
Indigenous	Tomato	Kathmandu, Nepal	+	+

Present (+), Absent (-).

Table 3. Profile of secondary endosymbiotic bacteria in different foreign whiteflies on various host plants from different countries.

Species	Host plants	Locations	*Cardinium*
Trialeurodes vaporariorum	Tomato	Euiseong, Korea	+
T. vaporariorum	Tomato	Gimcheon, Korea	+
T. vaporariorum	Cucumber	Sangju, Korea	+
T. vaporariorum	Tomato	Kathmandu, Nepal	+
T. vaporariorum	Brinjal	Kathmandu, Nepal	+
T. vaporariorum	Unknown	Japan	-
T. vaporariorum	Unknown	New Zealand	-
Tetraleurodes acaciae	Unknown	Calamba, Philippines	-
Aleurodicus dispersus	Eggplant	Patuakhali, Bangladesh	-
A. dispersus	Guava	Dumki, Bangladesh	-
A. dispersus	Guava	Magway, Myanmar	+
A. dispersus	Unknown	Calamba, Philippines	+
A. dispersus	Unknown	Calamba, Philippines	+

Present (+), Absent (-)

Phylogenetic analysis of *Cardinium*

Neighbour-joining phylogenetic tree reconstructed based on nineteen 16S rDNA sequences of *Cardinium*, which was detected in *B. tabaci*, *T. vaporariorum*, and *A. dispersus* from various countries, is shown in Table 2 and 3. The results showed that the distribution of *Cardinium* endosymbiont was highly diverse due to host and geographical variations. We observed high genetic variance among the 16S rDNA sequences of *Cardinium* from different countries (Fig. 1) and established four distinct clades, namely C1, C2, C3 and C4. Diversified endosymbiont identification for *Cardinium* was performed by sequencing the PCR products of individuals originating from Bangladesh, Myanmar, Nepal, the Philippines, and Republic of Korea (Fig. 1).

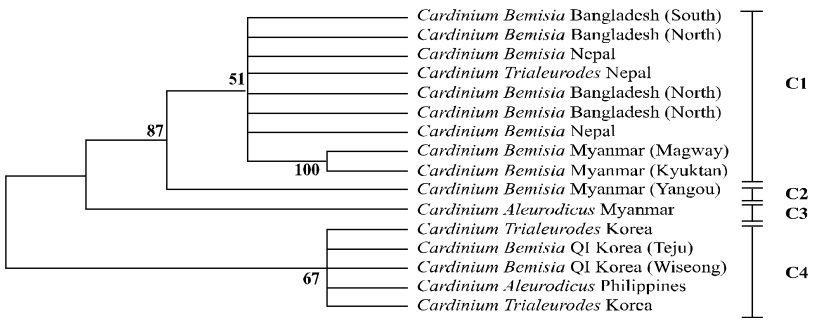

Figure 1. Phylogenetic relationship of 16S rDNA sequences of *Cardinium* in *B. tabaci* were compared with other *Cardinium* in different whitefly. According to the Bayesian method, the neighbor-joining (NJ) tree based on a fragment (~400 bp) using Kimura 2-parameter distances with complete deletion of gap/missing data, by partial 16S rDNA sequences. The number on each branch is the bootstrap support (1,000 replicates).

Analysis of 16S rDNA gene sequences of *Cardinium*

Sixteen sequences of 16S rDNA gene of *Cardinium* in different whiteflies from different countries were analyzed. These sequences had shown the proportions of A+T and G+C in residues composition 48.4% and 51.6% respectively. The average proportion of T: C: A: G was 22.4: 21.4: 25.9: 30.2 with a narrow standard error around means, but base composition varied substantially in different portions within the sequences of species. Among these 311 bp nucleotide, 249 characters were conserved, 62 characters were variable and 30 characters were singleton for parsimony analysis (Table 5). The sequence divergence in pairwise comparisons revealed that *Cardinium* is also very diverse group of endosymbiont like *Arsenophonus* ie. much divergence make 4 clades (C1 – C4) in phylogenetic tree where number of nucleotide changed 1-50 from each other, and distance of value was lowest 0.003 and highest 0.182 among all examined *Cardinium* sequences. *Cardinium* in *B. tabaci* from Bangladesh (BW1 and BW2), Myanmar (Magway and Kyuktan), Nepal and also from *T. vaporariorum* from Nepal make a single clade C1 in the tree (Figs. 1 and 2), *Cardinium* in *B. tabaci* from Myanmar (Yangon) makes another clade C2, *A. dispersus* from Myanmar (Magway) makes C3, and the 4th clade C4 makes by *Cardinium* in Q1 biotype of *B. tabaci* from Korea, *T. vaporariorum* from Korea and *A. dispersus* from the Philippines (Fig. 1 and Table 4). The genetic relationship among *Cardinium* sequences were extracted from neighbor-joining method (NJ). Analysis ran with Kimura's 2-parameter distance model using the Mega 4 program. The inferred phylogenetic topology based on NJ tree was diversified to each other. Result showed that *Cardinium* is divergent due to geographical barrier not depends on host. *Cardinium* makes same clade even though harbored in different species (different hosts).

(*Cardinium* in *B. tabaci* from Nepal (1 and 6), Korea (4 and 8), Myanmar (5, 7 and 16), Bangladesh (11, 13, 14 and 15); in *Aleurodicus dispersus* from Myanmar (2), the Philippines (3); in *Trialeurodes vaporariorum* from Korea (9 and 12), Nepal (10)). Distance between 16S rDNA gene for *Cardinium* (below diagonal: total nucleotide differences, above diagonal: mean character differences) using Kimura 2-parameter.

Indigenous Bangla	TGGAAGGTCCCCCACACTGGCACTGAGATACGGGCCAGACTCCTACGGGAGGCAGCAGTA	60
Indigenous Myanmar	TGGAAGGTCCCCCACACTGGCACTGAGATACGGGCCAGACTCCTACGGGAGGCAGCAGTA	60
Indigenous Nepal	TGGAAGGTCCCCCACACTGGCACTGAGATACGGGCCAGACTCCTACGGGAGGCAGCAGTA	60
Q-biotype Korea	TGGAAGATCCCCCACACTGGCACTGAGATACGGGCCAGACTCCTACGGGAGGCAGCAGTA	60
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Indigenous Bangla	GGGAATATTGGTCAATGGGCGCAAGCCTGAACCAGCCATGCCGCGTGCAGGATGAAGGCT	120
Indigenous Myanmar	GGGAATATTGGTCAATGGGCGCAAGCCTGAACCAGCCATGCCGCGTGCAGGATGAAGGCT	120
Indigenous Nepal	GGGAATATTGGTCAATGGGCGCAAGCCTGAACCAGCCATGCCGCGTGCAGGATGAAGGCT	120
Q-biotype Korea	GGGAATATTGGTCAATGGGCGAAAGCCTGAACCAGCCATGCCGCGTGCAGGATGACAGCT	120
	********** ****** ********** ***	
Indigenous Bangla	CTCTGAGTTGTAAACTGCTTTTGTACAGGAGCAAAAAAATCCCTGCGGGGGTTCTTGAGA	180
Indigenous Myanmar	CTCTGAGTTGTAAACTGCTTTTGTACAGGAGCAAAAAAATCCCTGCGGGGGTTCTTGAGA	180
Indigenous Nepal	CTCTGAGTTGTAAACTGCTTTTGTACAGGAGCAAAAAAATCCCTGCGGGGGTTCTTGAGA	180
Q-biotype Korea	CTCTGAGTTGTAAACTGCTTTTGTACAGGAGCAAAACAATCCCTGCGGGGGTTCTTGAGA	180
	********** ********** **********	
Indigenous Bangla	GTACTGTAAGAATAAGCACCGGCTAATTCCGTGCCAGCAGCCGCGGTAATACGGGAGGTG	240
Indigenous Myanmar	GTACTGTAAGAATAAGCACCGGCTAATTCCGTGCCAGCAGCCGCGGTAATACGGGAGGTG	240
Indigenous Nepal	GTACTGTAAGAATAAGCACCGGCTAATTCCGTGCCAGCAGCCGCGGTAATACGGGAGGTG	240
Q-biotype Korea	GTACTGTAAGAATAAGCACCGGCTAATTCCGTGCCAGCAGCCGCGGTAATACGGAAGGTG	240
	********** *****	
Indigenous Bangla	CAAGCGTTATCCGGTTTTATTGGGTTTAAAGGGTGCGTAGGCGGCTTATTAAGTCAGTTG	300
Indigenous Myanmar	CAAGCGTTATCCGGTTTTATTGGGTTTAAAGGGTGCGTAGGCGGCTTATTAAGTCAGTTG	300
Indigenous Nepal	CAAGCGTTATCCGGTTTTATTGGGTTTAAAGGGTGCGTAGGCGGCTTATTAAGTCAGTTG	300
Q-biotype Korea	CAAGCGTTATCCGGTTTTATTGGGTTTAAAGGGTGCGTAGGCGGCTTATTAAGTCAGTTG	300
	**********	
Indigenous Bangla	TGAAATCCTAGTGCTTAACGCTAGAACT	328
Indigenous Myanmar	TGAAATCCTAGTGCTTAACGCTAGAACT	328
Indigenous Nepal	TGAAATCCTAGTGCTTAACGCTAGAACT	328
Q-biotype Korea	TGAAATCCTAGTGCTTAACGCTAGAACT	328
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Fig. 2. Sequence alignments of *Cardinium* in different biotypes of *B. tabaci* from different countries using partial 16S rDNA gene sequences (5'-3') by the ClustalW2 program.

Table 4. Pairwise distance among 16 *Cardinium* endosymbiont in various whiteflies from different countries based on sequences of the fragment of 16S rDNA gene.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16]
[1]		0.020	0.108	0.020	0.085	0.000	0.085	0.043	0.023	0.000	0.000	0.020	0.000	0.000	0.003	0.010
[2]	6		0.093	0.020	0.104	0.020	0.104	0.043	0.020	0.020	0.020	0.020	0.020	0.020	0.023	0.023
[3]	31	27		0.086	0.182	0.108	0.182	0.108	0.086	0.108	0.108	0.086	0.108	0.108	0.111	0.111
[4]	6	6	25		0.100	0.020	0.100	0.023	0.003	0.020	0.020	0.000	0.020	0.020	0.023	0.023
[5]	25	30	50	29		0.085	0.000	0.126	0.100	0.085	0.085	0.100	0.085	0.085	0.089	0.096
[6]	0	6	31	6	25		0.085	0.043	0.023	0.000	0.000	0.020	0.000	0.000	0.003	0.010
[7]	25	30	50	29	0	25		0.126	0.100	0.085	0.085	0.100	0.085	0.085	0.089	0.096
[8]	13	13	31	7	36	13	36		0.026	0.043	0.043	0.023	0.043	0.043	0.047	0.047
[9]	7	6	25	1	29	7	29	8		0.023	0.023	0.023	0.023	0.023	0.026	0.026
[10]	0	6	31	6	25	0	25	13	7		0.000	0.020	0.000	0.000	0.003	0.010
[11]	0	6	31	6	25	0	25	13	7	0		0.020	0.000	0.000	0.003	0.010
[12]	6	6	25	0	29	6	29	7	1	6	6		0.020	0.020	0.023	0.023
[13]	0	6	31	6	25	0	25	13	7	0	0	6		0.000	0.003	0.010
[14]	0	6	31	6	25	0	25	13	7	0	0	6	0		0.003	0.010
[15]	1	7	32	7	26	1	26	14	8	1	1	7	1	1		0.013
[16]	3	7	32	7	28	3	28	14	8	3	3	7	3	3	4	

Table 5. Percentage of nucleotide frequencies in variable DNA sites of *Cardinium* endosymbiont in various whiteflies from different countries based on sequences of the fragment of 16S rDNA gene.

Cardinium	Nucleotide composition (%)				Conserved sites (%) (249/311)				Variable sites (%) (62/311)				Singleton sites (%) (30/311)				Total
	T	C	A	G	T	C	A	G	T	C	A	G	T	C	A	G	
Bt-N2	22.5	21.5	25.1	30.9	21.7	19.7	27.7	30.9	25.8	29.0	14.5	30.6	33.3	30.0	23.3	13.3	311
Ad-M	22.5	21.2	26.4	29.9	21.7	19.7	27.7	30.9	25.8	27.4	21.0	25.8	33.3	26.7	26.7	13.3	311
Ad-P	23.9	21.9	25.5	28.7	21.7	19.7	27.7	30.9	32.8	31.1	16.4	19.7	43.3	33.3	13.3	10.0	310
Bt-K1	22.5	21.9	25.7	29.9	21.7	19.7	27.7	30.9	25.8	30.6	17.7	25.8	33.3	30.0	23.3	13.3	311
Bt-M16	21.2	19.9	29.6	29.3	21.7	19.7	27.7	30.9	19.4	21.0	37.1	22.6	33.3	30.0	23.3	13.3	311
Bt-N7	22.5	21.5	25.1	30.9	21.7	19.7	27.7	30.9	25.8	29.0	14.5	30.6	33.3	30.0	23.3	13.3	311
Bt-M17	21.2	19.9	29.6	29.3	21.7	19.7	27.7	30.9	19.4	21.0	37.1	22.6	33.3	30.0	23.3	13.3	311
Bt-K2	22.5	21.9	25.7	29.9	21.7	19.7	27.7	30.9	25.8	30.6	17.7	25.8	33.3	30.0	23.3	13.3	311
Tv-K1	22.8	21.5	25.7	29.9	21.7	19.7	27.7	30.9	27.4	29.0	17.7	25.8	33.3	30.0	23.3	13.3	311
Tv-N	22.5	21.5	25.1	30.9	21.7	19.7	27.7	30.9	25.8	29.0	14.5	30.6	33.3	30.0	23.3	13.3	311
Bt-BW1	22.5	21.5	25.1	30.9	21.7	19.7	27.7	30.9	25.8	29.0	14.5	30.6	33.3	30.0	23.3	13.3	311
Tv-K2	22.5	21.9	25.7	29.9	21.7	19.7	27.7	30.9	25.8	30.6	17.7	25.8	33.3	30.0	23.3	13.3	311
Bt-BW2	22.5	21.5	25.1	30.9	21.7	19.7	27.7	30.9	25.8	29.0	14.5	30.6	33.3	30.0	23.3	13.3	311
Bt-M18	21.9	21.9	25.4	30.9	21.7	19.7	27.7	30.9	22.6	30.6	16.1	30.6	26.7	33.3	23.3	16.7	311
Avg.	22.4	21.4	25.9	30.2	21.7	19.7	27.7	30.9	25.4	28.4	18.8	27.4	33.8	30.0	22.9	13.3	310.9

(*Cardinium* in *B. tabaci* from Nepal (Bt-N2 and Bt-N7), Korea (Bt-K1 and Bt-K2), Myanmar (Bt-M16, Bt-M17 and Bt-M18), Bangladesh (Bt-BW1 and Bt-BW2); in *Aleurodicus dispersus* from Myanmar (Ad-M), the Philippines (Ad-P); in *Trialeurodes vaporariorum* from Korea (Tv-K1 and Tv-K2), Nepal (Tv-N))

Discussion

This study was attempted to identify and analyze *Cardinium* infection in different hosts from Bangladesh, Myanmar, Nepal, Republic of Korea and the Philippines. The presence of *Cardinium* was very consistent among indigenous genetic groups of *B. tabaci*, Q1 biotype of *B. tabaci* and *T. acaciae*, whereas it was rarely present in *T. vaporariorum* from Korea and Nepal and *A. dispersus* from Myanmar and Philippines but not in Bangladesh. In support of our results, high rates of *Cardinium* infection were reported in invasive and indigenous biotypes as well as Q1 biotype of *B. tabaci* from Korea (Jahan *et al*., 2011; Park *et al*., 2012).

The presence of *Cardinium* was not consistent among *B. tabaci* populations. *Cardinium* was absent in *B. tabaci* both B and Q biotype from Israel, whereas it was present in MS and Q1 biotype (Chiel *et al*., 2007). Gueguen *et al*. (2010) also showed that *Cardinium* was present in Q1 and MS biotypes, but not in B, Q2 or Q3 biotype. Our results further show that *Cardinium* infection was very common in all collected indigenous biotype populations from different countries, except for B biotype from Korea.

Our sequence analysis shows that *Cardinium* deviated into four genotypic groups which clustered in individual clades, each having a different host. Recently, a high level of genetic diversity was reported for *Wolbachia*, with 36 unique strains detected by Ros *et al*. (2012) and using sequences analysis Jahan and Lee, (2012) reported that 4 monophyletic clades of endosymbiont *Wolbachia* found in examined whitefly population. Similarly, 19 allelic profiles and six phylogenetic groups were obtained for the endosymbiont *Arsenophonus* among 152 individuals, demonstrating this bacterium's high diversity (Mouton *et al*., 2012). *Wolbachia* and *Cardinium* have been found to co-infect the same host species (Duron *et al*., 2008).

Conclusion

The present study shows that all of the *B. tabaci* population collected from different host plants in Bangladesh, Myanmar, and Nepal were infected by *Cardinium*, whereas the B biotype from Korea and Q biotype population from China was not infected. According to the 16S rDNA sequence analysis, we identified four phylogenetic clades, illustrating the divergence of *Cardinium* endosymbiont.

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