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Article

# Upregulation of Endosymbiont Densities in *Bemisia tabaci* by Acquisition of *Tomato Yellow Leaf Curl Virus*

S. M. Hemayet Jahan, Kyeong-Yeoll Lee*
School of Applied Biosciences, Kyungpook National University, Daegu 702-701, Republic of Korea

## Abstract

Sweetpotato whitefly, *Bemisia tabaci*, is a vector of more than 100 plant-diseased viruses, as well as a serious pest of various horticultural plants. This species harbors a primary endosymbiont *Portiera* along with several secondary endosymbionts such as *Cardinium* and *Hamiltonella*. We investigated whether or not TYLCV acquisition alters the densities of endosymbionts in the body of *B. tabaci* using quantitative real-time PCR. Our results showed that the densities of both *Cardinium* and *Hamiltonella*, but not *Portiera*, increased upon acquisition of TYLCV. In addition, expression of GroEL, a molecular chaperone produced by *Hamiltonella*, was significantly upregulated in TYLCV-infected whiteflies. Our results suggest that endosymbionts may play an important role in TYLCV transmission mechanism within the body of *B. tabaci*.

**Keywords** : *Cardinium*, GroEL, *Hamiltonella*, TYLCV, Vector insects, Virus transmission

## Introduction

Endosymbiotic bacteria have been identified in various arthropod species including insects. It has become increasingly evident that endosymbionts can have major effects on their host's biology, ecological relationships, and evolutionary dynamics (Moran, 2006). The influence of endosymbionts on their hosts can range from beneficial to detrimental, and effects may be conditional on extrinsic factors (Russell and Moran, 2006; Haine, 2008).

*Bemisia tabaci* harbors primary obligate and secondary facultative endosymbionts (Baumann, 2005). The primary endosymbiont *Portiera* is harbored within bacteriocytes and supplements its insect host with essential amino acids for growth and reproduction (Douglas, 1989; Baumann et al., 2000 Moran et al., 2003). In addition, *B. tabaci* harbors several secondary endosymbionts, including *Arsenophonus* (Thao and Baumann, 2004), *Cardinium* (Weeks et al., 2003), *Fritschea* (Everett et al., 2005), *Hamiltonella* (Moran et al., 2005), *Rickettsia* (Gottlieb et al., 2006), and *Wolbachia* (Zchori-fein and Brown, 2002). In addition to providing essential nutrients to their insect hosts, these endosymbionts bestow functional capabilities such as temperature tolerance (Montlloret al., 2002), increased resistance to parasites (Oliver et al., 2003), increased resistance to insecticides (Kontsedalov et al., 2008), sex determination (Himler et al., 2011), and other unidentified physiological and ecological activities.

Endosymbionts play a major role in virus transmission of *B. tabaci* as well as other vector insects. Several studies have shown that a molecular chaperone, GroEL protein, produced from endosymbionts has a protective role by binding to coat protein of viruses in the vector's body. GroEL protein of *Buchnera aphidicola* binds to luteovirus coat protein and prevents virus particles from proteolysis in the gut and hemolymph of *Myzus persicae* (Van den Heuvel et al., 1997). It has also been reported that interrupting the interaction between GroEL and coat protein leads to a dramatic decrease in virus transmission efficiency in *B. tabaci* (Morin et al., 1999, 2000). *Tomato yellow leaf curl virus* (TYLCV) is exclusively vectored by *B. tabaci*, and its transmission depends on the presence of GroEL protein, which is produced by *Hamiltonella* but not other endosymbionts (Gottlieb et al., 2010). Further, Rana et al. (2012) reported that *Cotton leaf curl virus* (CLCuV) coat protein strongly interacts with GroEL from *Arsenophonus*, whereas both *Escherichia coli* and *Portiera* GroELproteins do not interact with CLCuV coat protein. They suggest *Arsenophonus* is highly involved in the transmission of CLCuV in the AsiaII genetic group of *B. tabaci*. Therefore, it is likely that the relationship between virus and endosymbiont is highly specific at a species level within the vector's body. Here, we demonstrated whether TYLCV acquisition influence on the density of different endosymbionts

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*Corresponding Author: Kyeong-Yeoll Lee, Tel. 82-53-950-5759, Fax. 82-53-950-6758, Email. leeky@knu.ac.kr
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in *B. tabaci*.

## Materials and methods

### Insect collection and rearing

*Bemisia tabaci* were initially collected from Korean melon (*Cucumis melo* var. *makuwa*) grown in a greenhouse in Seongju, Korea in 2009 and maintained in the laboratory as a non-viruliferous (NV) colony on tomato plants (*Lycopersicon esculentum* Mill.) in insect-proof cages (45 × 60 × 90 $cm^3$). A portion of the NV colony was transferred into another cage housing TYLCV-infected tomato plants and maintained as a TYLCV-viruliferous (V) colony. These two colonies were then reared in separate insect-rearing rooms under conditions of 25 ± 2℃, 60 ± 5% relative humidity, and a 16 h light/8 h dark (16L:8D) photoperiodic cycle.TYLCV infection of both colonies was confirmed by PCR analysis at regular intervals over 1 month (Park et al., 2012). After every month, fresh TYLCV-free tomato plants were put into both cages. Our previous study on the identification of biotype and endosymbiont profile shows that our colony is Q1 biotype (mtCOI GenBank accession number HM488315) (Park et al., 2012).

### Clip cage assay

Clip cage was a hand-made instrument, namely a crystal clear, plastic, round-shaped tube (1.5 cm diameter). Specifically, a tube was cut to a length of 0.5 cm and sealed at one side with a fine insect-proof mesh. A small-sized hole (2 mm diameter) was prepared on the side of the cage in order to allow whiteflies to enter. A lid was prepared from a plastic piece having the same size as the tube, after which both the cage and lid were attached to the clip. The clip cage was then attached to the underside of a tomato plant leaf. To make TYLCV-infected tomato plants, adult V whiteflies (n=50) were collected from the V colony using a fine glass tube (act as an aspirator) and aspired into the clip cage through the hole, after which they were kept in a mesh acrylic glass box (30×30×40 $cm^3$) for up to 48 hours (Fig. 1A). After 48 hours, we removed the clip cage and collected a small part of the leaves in order to confirm TYLCV infection by PCR (Fig. 1B). The leaves were then put into an acrylic box and were considered as TYLCV-infected tomato plants. We used another box to house TYLCV-free tomato plants. Subsequently, we released the same number (n=500) of NV day-0 whiteflies in the two acrylic cages. Every conjugative day (2, 4, 6, and 8), we collected the same number of whiteflies from both cages for DNA and RNA extraction (25 for DNA and 100 for RNA extraction).

### Genomic DNA extraction

Total genomic DNA was extracted from 25 individual adult *B. tabaci* whiteflies according to the protocol supplied by the Invitrogen Purelink Genomic DNA mini kit. After collecting

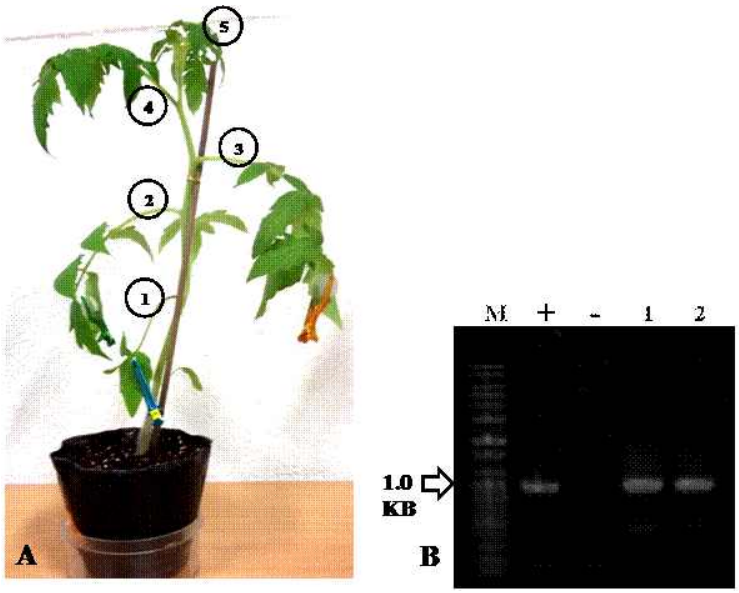


**Fig 1. Procedure for construction of TYLCV-infected tomato plants using clip cages with viruliferous whitefly.**

Five leaves (1-5) of tomato plant prepared with three clip cages, which were attached to the 1st, 2nd, and 3rd leaves (A). After 48 hours, TYLCV infection was checked using small pieces from the 4th and 5th leaves by PCR (B). DNA marker (M), a positive control (+), a negative control (-), 4th leaf (1), and 5th leaf (2).

the samples from individual NV and V colonies, whiteflies were homogenized in 180 μl of genomic digestion buffer using a 1.5 ml microcentrifuge tube and micropestle (homogenizer). After the addition of 200 μl of genomic lysis/binding buffer (1% SDS, 10 mM Tris-HCl, pH 8.0, 25 mM EDTA, 25 mM NaCl, 200 mg/ml of Proteinase K), 200 μl of absolute ethanol was immediately added. Wash buffer was then added into the genomic column, after which 20 μl of genomic elusion buffer (Invitrogen Purelink, USA) was added. After 1 min of incubation at room temperature, samples were centrifuged at about 12,000 rpm for 1 min. The supernatants and pellets were directly used for PCR detection of secondary endosymbionts or stored at -20℃ for later use.

**cDNA production**

Total RNAs were extracted from whole bodies of 100 adult *B. tabaci* Q1 biotype NV and V whiteflies that were 2, 4, 6, and 8 days old (D2, D4, D6, and D8, respectively) according to the protocol with TRI reagent (MRC Inc., Cincinnati, OH, USA.). All extracts were treated with RQ1 DNAase (RNase-free) (Promega, Madison, WI, USA) to avoid DNA contamination. RNAs were subjected to gel electrophoresis and quantified using an IMPLEN Nano photometer (Implen GmbH, Munich, Germany). Two micrograms of RNA was used to synthesize cDNA using a High capacity cDNA reverse transcription kit (Applied Biosystems, Foster city, CA, USA) in a total reaction volume of 20 μl.

**Quantitative real-time polymerase chain reaction (qRT-PCR)**

Quantification of the different endosymbionts was performed by amplification using Power SYBR Green PCR Master Mix (Applied Biosystems, USA) and 10 pmol of each primer. Using nucleotide sequences from the NCBI database, gene-specific primers for TYLCV, *Portiera* (primary endosymbiont) 16S rDNA, *Portiera* GroEL, *Cardinium* (secondary endosymbiont) 16S rDNA, *Hamiltonella* (secondary endosymbiont) 16S rDNA, *Hamiltonella* GroEL, and *B. tabaci* actin were designed for quantitative real-time PCR (Table 1). The cDNA samples (0.2 μl) were run in triplicate on a 7300 Sequence Detection System (Applied Biosystems, USA) for 1 cycle of denaturation (95℃ for 15 min), 45 cycles of amplification (95℃ for 10 sec; 58℃ for 20 sec; 72℃ for 30 sec), and 1 cycle of dissociation (95℃ for 15 sec; 60℃ for 30 sec; 95℃ for 15 sec). The expression level of each gene was determined by measuring the relative quantities of cDNAs to their respective mRNAs. The Ct (Threshold cycles) values were used to calculate the mRNA levels. Data were analyzed using the formula: $2^{-\triangle\triangle Ct} = 2^{-[\triangle Ct\ treatment - \triangle Ct\ control]}$. The partial nucleotide sequence of actin from *B. tabaci* (AF071908) was identified from the *Bemisia* EST database in NCBI Genbank. The actin level was used as a reference to normalize the expression levels of the other genes. To ensure validity of the data, the number of bacterial genomes was tested in triplicate in each independent experiment.

**Table 1. Oligonucleotide primer sequences used in quantitative real-time PCR**

| Targeted gene | Primer sequence (5' to 3') | Product size (bp) |
|---|---|---|
| TYLCV | F-TCTGTTCACGGATTTCGTTG<br>R-GCTGTCGAAGTTCAGCCTTC | 182 |
| *Portiera* 16S rRNA | F-TCAACTAGCCGTTGGATTCTTAAA<br>R-TACTCCCCAGGCGATCAACT | 71 |
| *Cardinium* 16S rRNA | F-GCCGGCGACCGGCGAATG<br>R-CGGAGGCTATTCCCCAGTGT | 66 |
| *Hamiltonella* 16S rRNA | F-GTAAACGATGTCGATTTGGAGGT<br>R-GCGGTCGATTTAACGCGT | 71 |
| *Portiera* GroEL | F-GTTGTAGCTGGAGGAGGTACTGCTT<br>R-TGTTTGGTCTTCGTTGTTGCC | 71 |
| *Hamiltonella* GroEL | F-TCTTCGATTTTTCACCTGCTCC<br>R-CAATGCAAAACGCGTCGTC | 71 |
| *B. tabaci* Actin | F-GACGGACAGGTCATCATAATCG<br>R-CATACCCAAGAAGGATGGCTG | 78 |
| *B. tabaci* Actin | F-TCA CCA CCA CAG CTG AGA GA<br>R-CTC GTG GAT ACC GCA AGA TT | 231 |

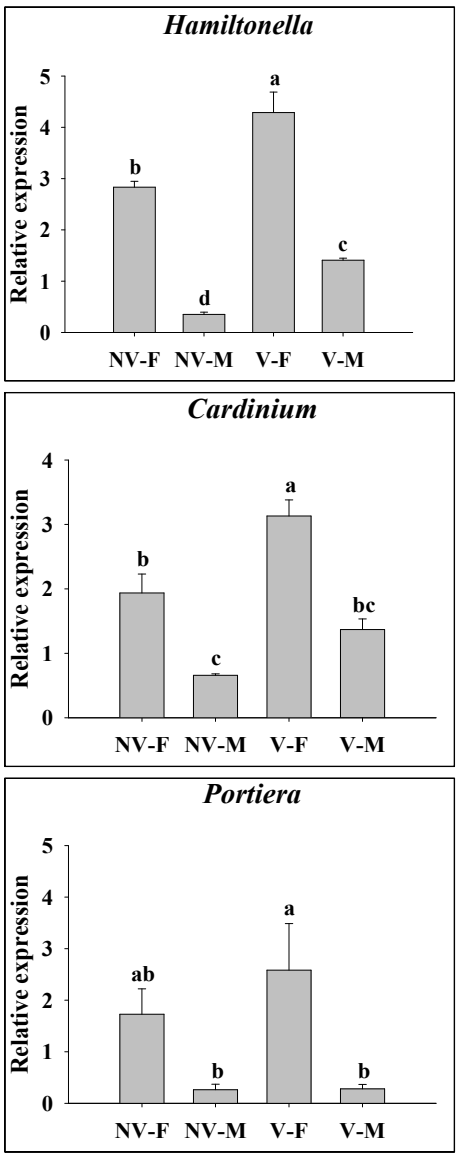


**Fig 2. Quantitative real-time PCR analysis of endosymbiont density.**

Genomic DNA samples were extracted from female (NV-F) and male (NV-M) of non-viruliferous individuals, and female (V-F) and male (V-M) of viruliferous individuals of *B tabaci* (n = 100). Different letters above columns indicate statistically significant differences (P < 0.05) using DMRT by SAS.

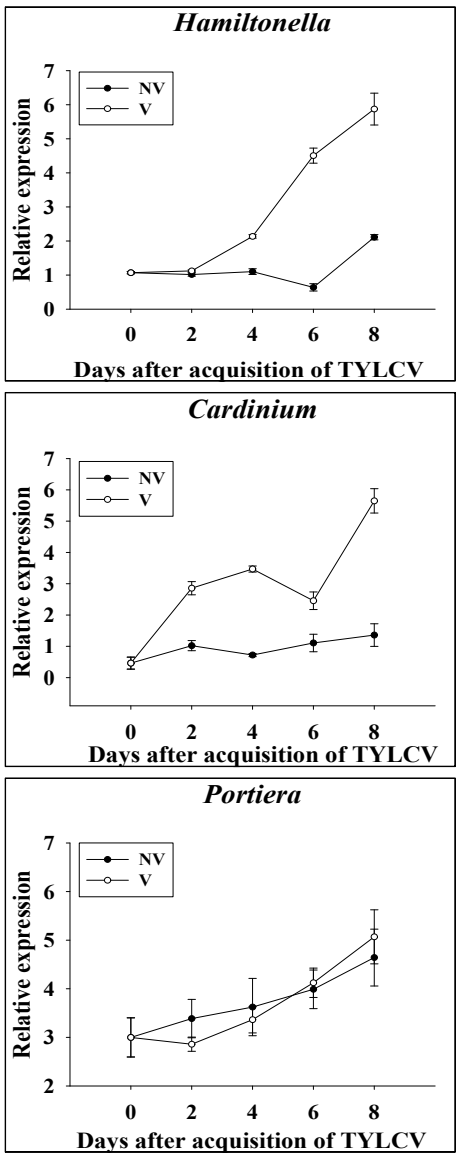


**Fig 3. Comparison of densities of three endosymbionts between NV and V *B. tabaci*.**

Quantitative real-time PCR analysis was conducted using genomic DNA samples collected from adult *B tabaci* of 0, 2, 4, 6, and 8 days after TYLCV acquisition.

### Statistical analysis

Data were expressed in three individual replicates, and mean values ± standard error were plotted using Sigma plot (Systat software, Inc., Point Richmond, CA, USA). Analysis of variance (ANOVA) was carried out in order to analyze the means by using PROC General Linear Model (GLM) with the Statistical Analysis System (SAS, 2002-2003 SAS Institute Inc., Cary, NC, USA) version 9.1 program. Significant differences among mean values were determined using Duncan's Multiple Range Tests (DMRT) at a 95% confidence level. Data were analyzed by using acompletely randomized design with three individual replications.

## Results

### Comparison of endosymbiont densities between NV and V whiteflies

Our previous study on the identification of biotype and

endosymbiont profile shows that our colony is Q1 biotype (mtCOI GenBank accession number HM488315) harboring the secondary endosymbionts *Cardinium* and *Hamiltonella*, but not *Arsenophonus, Rickettsia*, and *Wolbachia* (Park et al., 2012). Thus, we analyzed one primary (*Portiera*) and two secondary (*Cardinium and Hamiltonella*) endosymbionts.

Densities of primary (*Portiera*) and secondary (*Cardinium* and *Hamiltonella*) endosymbionts were significantly different between sexes (Fig. 2). Female whiteflies displayed higher densities of all three endosymbionts compared to males regardless of TYLCV acquisition. In comparing NV with V whiteflies, endosymbiont densities mostly increased in V whiteflies, although the rates slightly differed. For example, the density of *Portiera* slightly increased only in females, whereas the densities of both *Cardinium* and *Hamiltonella* significantly increased in both sexes (Fig. 2). When bacterial densities were analyzed continuously for 8 days, *Portiera* density gradually increased in both NV and V whiteflies. However, both *Cardinium* and *Hamiltonella* densities gradually increased in V whiteflies, whereas they remained at constant levels in NV whiteflies (Fig. 3).

**GroEL expression of endosymbionts in NV and V whiteflies**

To compare GroEL expression levels between NV and V whiteflies, adult whiteflies (n=100) were collected from NV and V colonies, after which GroEL mRNA levels were determined using quantitative real-time PCR analysis (Fig. 4). GroEL mRNA level of *Portiera* was gradually increased in both NV and V whiteflies. On the other hand, GroEL mRNA level of *Hamiltonella* was increased in V whiteflies from 2 days following TYLCV acquisition, whereas it remained at constant levels in TYLCV-free whiteflies.

## Discussion

Similar to a previous report, B and Q1 biotypes of *Bemisia tabaci* are present in Korea. The Q1 biotype is available everywhere in Korea, even Jeju-do island, whereas the B biotype is only restricted to the Goyang region (Park et al., 2012). Both B and Q1 biotypes of *B. tabaci* harbor various secondary endosymbionts with distribution patterns that vary according to biotype. For instance, *Hamiltonella* is common in both B and Q1 biotypes in Korea, whereas other endosymbionts are more selective (Park et al., 2012).

Here, we showed that the densities of both primary (*Portiera*) and secondary (*Cardinium* and *Hamiltonella*) endosymbionts were higher in female compared to male whiteflies. Our results are similar with those of Cohen and Harpaz (1964) and Muniyappa et al. (2000), who observed that virus diffusion of female whiteflies is higher than males in frequency upon virus transmission from infected to non-infected plants. Namely, females can transmit TYLCV about 5-fold more proficiently than males (Cohen and Harpaz, 1964). Similarly, ToLCV from Bangalore is transmitted by females more efficiently than by males (Muniyappa et al., 2000). Thus, endosymbiont density is sexually differentiated and this is highly significant in virus transmission of vector insects.

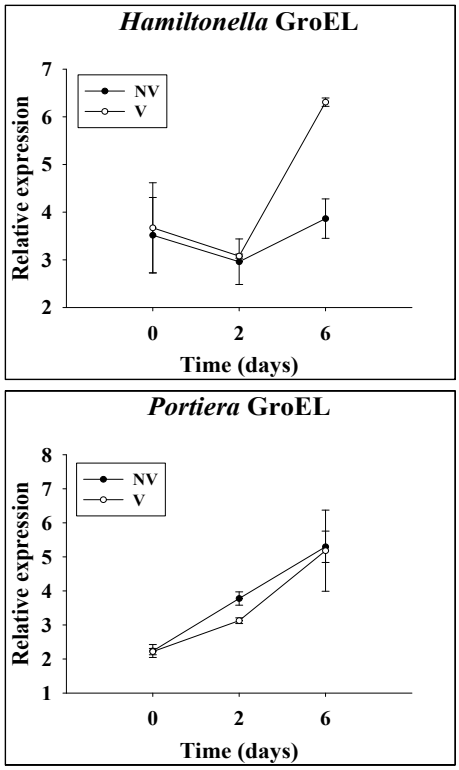


**Fig 4. Comparison of GroEL mRNA levels of endosymbionts between NV and V individuals of adult *B. tabaci*.**

Quantitative real-time PCR analysis was conducted using cDNA samples collected from 0, 2, and 6 day-old adult whiteflies.

Our genomic DNA analysis showed that endosymbiont densities were increased in both sexes of *B. tabaci* after acquisition of TYLCV. Particularly, this was significant in *Cardinium* and *Hamiltonella* endosymbionts. Otherwise, *Portiera* density was increased at a similar level in both NV and V individuals. Furthermore, GroEL mRNA level of *Hamiltonella* was increased upon TYLCV acquisition, whereas it remained steady in TYLCV-free whiteflies. Our results suggest that TYLCV acquisition induces multiplication of both *Cardinium* and *Hamiltonella* within vector's body. *Hamiltonella* is responsible

to protect TYLCV in the hemolymph by releasing GroEL which is bind to coat protein of TYLCV (Gottlieb et al., 2010). TYLCV particles may be more stable in the hemolymph due to the increased amount of *Hamiltonella*. We suppose that this mechanism is highly efficient for the successful transmission of virus.

Similar to *Hamiltonella*, we found that density of *Cardinium* was also increased by the acquisition of TYLCV. This suggests that *Cardinium* may also associate with transmission of TYLCV. However, there is no report on the relationship between *Cardinium* and virus transmission. *Cardinium* is usually located within the reproductive tissues and highly involved in reproduction alteration of various arthropod species (Zchori-Fein and Perlman, 2004). It is also possible that increased *Cardinium* by TYLCV acquisition may influence on the reproductive changes such as cytoplasmic incompatibility, parthenogenesis and feminization (Chigira and Miura, 2005).

In summary, our studies demonstrated that Q1 biotypes of *B. tabaci* increase densities of both *Cardinium* and *Hamiltonella* by the acquisition of TYLCV.We further determined that GroEL expression level was increased in *Hamiltonella*. These results suggest that virus vectoring ability of whiteflies could be increased by upregulated densities of the endosymbionts in viruliferous whiteflies.

## Acknowledgements

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## References

Baumann L, Thao ML, Funk CJ, Falk BW, Ng JCK, Baumann P (2004) Sequence analysis of DNA fragments from the genome of the primary endosymbiont of the whitefly *Bemisia tabaci*. *Current Microbiology* 48: 77-81.

Baumann P (2005) Biology bacteriocyte-associated endosymbionts of plant sap sucking insects. *Annual Review of Microbiology* 59: 155-189.

Baumann P, Moran NA, Baumann L (2000) Bacteriocyte-associated endosymbionts of insects. In: Dworkin, M. (ed.) The prokaryotes. *Springer*, New York.

Bouvaine S, Boonham N, Douglas AE (2011) Interactions between a luteovirus and the GroEL chaperonin protein of the symbiotic bacterium *Buchnera aphidicola* of aphids. *Journal of General virology* 92: 1467-1474.

Chiel E, Gottlieb Y, Zchori-Fein E, Mozes-Daube N, Katzir N, Inbar M, Ghanim M (2007) Biotype-dependent secondary symbiont communities in sympatric populations of Bemisia tabaci. *Bulletin of Entomological Research* 97: 407-413.

Chigira A, Miura K (2005) Detection of 'candidatus *Cardinium*' bacteria from the haploid host *Brevipalpus californicus* (Acari: Tenuipalpidae) and effect on the host. *Experimental AppliedAcarology* 37: 107-16.

Cohen S, Harpaz I (1964) Periodic, rather than continual acquisition of a new tomato virus by its vector, the tobacco whitefly (*Bemisia tabaci* Gennadius). *Entomologia Experimentalis et Applicata* 7: 155-166.

Czosnek H, Ghanim M, Ghanim M (2002) Circulative pathway of begomoviruses in the whitefly vector *Bemisia tabaci* - insights from studies with *Tomato yellow leaf curl virus*. *Annals of Applied Biology* 140: 215-231.

Douglas AE (1989) Mycetocyte symbiosis in insects. *Biological reviews of the Cambridge Philosophical Society* 64: 409-434.

Everett KDE, Thao M, Horn M, Dyszynski GE, Baumann P (2005) Novel chlamydiae in whiteflies and scale insects: endosymbionts 'Candidatus *Fritschea bemisiae*' strain Falk and 'Candidatus *Fritschea eriococci*' strain Elm. *International Journal of Systematic and Evolutionary Microbiology* 55: 1581-1587.

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

Gottlieb Y, Zchori-Fein E, Mozes-Daube N, Kontsedalov S, Skaljac M, Brumin M, Sobol I, Czosnek H, Vavre F, Fleury F, Ghanim M (2010) The transmission efficiency of *Tomato yellow leaf curl virus* by the whitefly *Bemisia tabaci* is correlated with the presence of a specific symbiotic bacterium species. *Journal of Virology* 84: 9310-9317.

Haine ER (2008) Symbiont-mediated protection. *Proceedings of the Royal Society of London. Series B, Biological Science* 275: 353-361.

Himler AG, Adachi-hagimori T, Bergen JE, Kozuch A, Kelly SE, Tabashnik BE, Chiel E, Duckworth VE, Dennehy TJ, Zchori-Fein E, Hunter MS (2011) Rapid spread of a bacterial symbiont in an invasive whitefly is driven by fitness benefits and female bias. *Science* 332: 254-256.

Kontsedalov S, Zchori-fein E, Chiel E, Gottlieb Y, Inbar M, Ghanim M (2008) The presence of *Rickettsiais* associated with increased susceptibility of *Bemisia tabaci* (Homoptera:

Aleyrodidae) to insecticides. *Pest Management Science* 792: 789-792.

Montllor CB, Maxmen A, Purcell AH (2002) Facultative bacterial endosymbionts benefit pea aphids *Acyrthosiphon pisum* under heat stress. *Ecological Entomology* 27: 189-195.

Moran NA (2006) Symbiosis (A primer). *Current Biology* 16: R866-871.

Moran NA, Degnan PH, Santos SR, Dunbar HE, Ochman H (2005) The players in a mutualistic symbiosis: insects, bacteria, viruses, and virulence genes. *Proceedings of the National Academy of Sciences of the United States of America* 102: 16919-16926.

Moran NA, Plague GR, Sandstrom JP, Wilcox JL (2003) A genomic perspective on nutrient provisioning by bacterial symbionts of insects. *Proceedings of the National Academy of Sciences of the United States of America* 100: 14543-14548.

Morin S, Ghanim M, Sobol I, Czosnek H (2000) The GroEL protein of the whitefly *Bemisia tabaci* interacts with the coat protein of transmissible and nontransmissible begomoviruses in the yeast two-hybrid system. *Virology* 276: 404-416.

Morin S, Ghanim M, Zeidan M, Czosnek H, Verbeek M, Heuvel JFJM (1999) A GroEL homologue from endosymbiotic bacteria of the whitefly *Bemisia tabaci* is implicated in the circulative transmission of *Tomato yellow leaf curl virus*. *Virology* 256: 75-84.

Muniyappa V, Venkatesh HM, Ramappa HK, Kulkarni RS, Zeidan M, Tarba CY, Ghanim M, Czosnek H (2000) *Tomato leaf curl virus* from Bangalore (ToLCV-Ban4): sequence comparison with Indian ToLCV isolates, detection in plants and insects, and vector relationships. *Archives of Virology* 145: 1583-1598.

Oliver KM, Russell JA, Moran NA, Hunter MS (2003) 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.

Park J, Jahan SMH, Song WG, Lee H, Lee YS, Choi HS, Lee KS, Kim CS, Lee S, Lee KY (2012) Identification of biotypes and secondary endosymbionts of *Bemisia tabaci* in Korea and relationships with the occurrence of TYLCV disease. *Journal of Asia-Pacific Entomology* 15: 186-191.

Rana VS, Singh ST, Priya NG, Kumar J, Rajagopal R (2012) *Arsenophonus* GroEL interacts with CLCuV and is localized in midgut and salivary gland of whitefly *B. tabaci*. *PLoS ONE* 7: e42168.

Russell JA, Moran NA (2006) Costs and benefits of symbiont infection in aphids: variation among symbionts and across temperatures. *Proceedings of the Royal Society B: Biological Sciences* 273: 603-610.

Thao ML, Baumann P (2004) Evolutionary relationships of primary prokaryotic endosymbionts of whiteflies and their hosts. *Applied and Environmental Microbiology* 70: 3401-3406.

Van den Heuvel JFJM, Bruye're A, Hogenhout SA, Ziegler-Graff V, Brault V, Verbeek M, Vanderwilk F, Richards K (1997) The N-terminal region of the luteovirus read through domain determines virus binding to *Buchnera* GroEL and is essential for virus persistence in the aphid. *Journal of virology* 71: 7258-7265.

Weeks AR, Velten R, Stouthamer R (2003) Incidence of a new sex-ratio distorting endosymbiotic bacterium among arthropods. *Proceedings of the Royal Society* B 270: 1857-1865.

Zchori-Fein AE, Brown JK (2002) Diversity of prokaryotes associated with *Bemisia tabaci* (Gennadius); (Hemiptera: Aleyrodidae). *Annals of the Entomological Society of America* 95 711-718.

Zchori-Fein AE, Perlman SJ (2004) Distribution of the bacterial symbiont *Cardinium* in arthropods. *Molecular Ecology* 13: 2009-2016.