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Identification and Localization of a *Rickettsia* sp. in *Bemisia tabaci* (Homoptera: Aleyrodidae)

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Whiteflies (Homoptera: Aleyrodidae) are sap-sucking insects that harbor "*Candidatus* Portiera aleyrodidarum," an obligatory symbiotic bacterium which is housed in a special organ called the bacteriome. These insects are also home for a diverse facultative microbial community which may include *Hamiltonella*, *Arsenophonus*, *Fritchea*, *Wolbachia*, and *Cardinium* spp. In this study, the bacteria associated with a B biotype of the sweet potato whitefly *Bemisia tabaci* were characterized using molecular fingerprinting techniques, and a *Rickettsia* sp. was detected for the first time in this insect family. *Rickettsia* sp. distribution, transmission and localization were studied using PCR and fluorescence in situ hybridizations (FISH). *Rickettsia* was found in all 20 Israeli *B. tabaci* populations screened but not in all individuals within each population. A FISH analysis of *B. tabaci* eggs, nymphs, and adults revealed a unique concentration of *Rickettsia* around the gut and follicle cells, as well as a random distribution in the hemolymph. We postulate that the *Rickettsia* enters the oocyte together with the bacteriocytes, leaves these symbiont-housing cells when the egg is laid, multiplies and spreads throughout the egg during embryogenesis and, subsequently, disperses throughout the body of the hatching nymph, excluding the bacteriomes. Although the role *Rickettsia* plays in the biology of the whitefly is currently unknown, the vertical transmission on the one hand and the partial within-population infection on the other suggest a phenotype that is advantageous under certain conditions but may be deleterious enough to prevent fixation under others.

The importance of symbionts as a molding force of arthropod biology is well established for primary symbionts, such as *Buchnera aphidicola* in aphids (4, 10), and accumulating data on secondary symbionts also suggest that these tenants play important roles in the biology of their hosts (e.g., see references 31 and 33).

Whiteflies are small homopterans that feed as nymphs and adults on the phloem sap of plants. The hatching crawler settles near the hatching site, where it goes through four immobile nymphal instars before developing into an adult. The sweet potato whitefly *Bemisia tabaci* (Gennadius) is a severe agricultural pest in many parts of the world (5). This species consists of several biotypes (3) that have been distinguished largely on the basis of biochemical or molecular diagnostics but whose biological significance is still unclear. Like other phloem-feeding insects, whiteflies require bacteria for supplementing their unbalanced diet. These symbionts are housed in specialized organs called bacteriomes, which are composed of bacteriocytes (2). "*Candidatus* Portiera aleyrodidarum," the primary symbiont of whiteflies, is an AT-rich member of the gamma subdivision of the *Proteobacteria* (2). In *B. tabaci*, "*Ca*. Portiera aleyrodidarum" is vertically transmitted through bacteriocyte inclusions into the oocyte at the point which will eventually become the pedicel end of the egg (9, 19).

Secondary symbionts of *B. tabaci* consist of a diverse array of bacteria which are phylogenetically related to other described symbionts of sap-feeding insects. Using transmission electron microscopy, Costa et al. (8) distinguished three types of secondary symbionts, two of which have been tentatively identified as "*Candidatus* Cardinium hertigii" (*Bacteriodetes*) (39) and *Fritchea bemisiae* (*Simkaniaceae*) (36), respectively. Sequence-based phylogenetic analysis of secondary symbionts found in various *B. tabaci* biotypes further revealed the presence of two enteric bacteria, one which resembles the aphid symbiont "*Candidatus* Hamiltonella defensa" and the other with high sequence similarity to the *Arsenophonus*-like psyllid symbiont (44) and to *Wolbachia* spp. (27).

A comprehensive characterization of the bacterial community in different *B. tabaci* populations is crucial for understanding various aspects of that pest's biology, such as the emergence of more aggressive biotypes and the variation in transmission capabilities of plant viruses (25). The research here aimed to profile the bacterial community found in one *B. tabaci* population. During the analysis, we identified a *Rickettsia bellii*-like bacterium, and since this is the first record of that bacterium in whiteflies, we further studied its spatial and temporal localization in various stages of whitefly development.

MATERIALS AND METHODS

Whitefly origin and rearing. *B. tabaci*, B biotype (pesticide-susceptible strain Ssc) was reared on cotton seedlings (Acala) under standard greenhouse conditions, at 26 ± 2°C, 60% relative humidity, and a photoperiod of 14 h of light and 10 h of darkness. This strain was collected from Israeli cotton fields during 1987

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TABLE 1. Israeli *B. tabaci* populations screened for the presence of *Rickettsia* sp.

B. tabaci population	% with *Rickettsia* sp. (total no. tested)	Collection site	Host plant	Collection date
ARV-03	100 (10)	Hazeva	Basil	June 2003
AV-99	90 (10)	Nachshon	Cotton	August 1999
AV-00	50 (10)	Nachshon	Cotton	August 2000
AV-01	90 (10)	Nachshon	Cotton	June 2001
AV-01	60 (10)	Nachshon	Cotton	August 2001
AV-02	90 (20)	Nachshon	Sunflower	June 2002
AV-02 Msp[a]	90 (20)	Nachshon	Cotton	August 2002
AV-03	22 (9)	Sha'albim	Sunflower	July 2003
BD-00	90 (10)	Bet Dagan	Cotton	July 2000
HC-01	90 (10)	Ma'yan Zevi	Cotton	August 2001
HC-02	60 (10)	Ma'yan Zevi	Cotton	August 2002
HC-03	81 (16)	Nahsholim	Cotton	July 2003
HC-03	47 (15)	Ma'yan Zevi	Cotton	September 2003
Neg-01	100 (10)	Bet Ha'Gaddi	Rose	August 2001
Neg-01	60 (10)	Nahal Oz	Cabbage	August 2001
Neg-02	89 (16)	Ashalim	Melon	July 2002
Neg-03	30 (10)	Nahal Oz	Broccoli	August 2003
Tgr-R[b]	94 (16)	Kefar Maimon	Rose	February 1992
Ssc-87	64 (17)	Zora	Cotton	August 1987
Ye-99	50 (20)	Yesha	Sweet potato	August 1999

[a] A line selected for "Mospilan" resistance (acetamiprid-neonicotinoid group).
[b] A line selected for "Tiger" resistance (pyriproxyfen-juvenile hormone analogue).

and has since been maintained in a closed laboratory culture without exposure to pesticides (Table 1).

PCR amplification and DGGE analysis. To establish the whole range of bacteria associated with the Ssc population, adults of *B. tabaci* were placed alive in 96% alcohol, and three replicates of one adult female each were ground in lysis buffer as described by Frohlich et al. (15). The 16S rRNA gene fragment (~550 bp) was amplified using PCR from the insect lysate using the primer combination of 341F with a GC clamp (40-nucleotide, GC-rich sequence) and 907R (Table 2), which targets most known *Bacteria*, with PCR conditions that permit its amplification from most known *Bacteria* (26). Reactions were performed in a 50-μl volume containing 5 μl of the template DNA lysate, 400 mM concentrations of each primer, 5 μl of 0.2 mM deoxynucleoside triphosphate, 1× ExTaq buffer, and 1 unit of ExTaq (TaKara Bio, Inc.). Five microliters of the PCR mix was tested using agarose gel electrophoresis, and the remaining 45 μl containing the amplified DNA fragments was then subjected to denaturing gradient gel electrophoresis (DGGE) analysis using the following conditions: separation using a 6% (wt/vol) acrylamide gel (acrylamide-*N*,*N*′-methylenebisacrylamide, 37.5:1) prepared in 1× Tris-acetate-EDTA buffer with a denaturing gradient ranging from 20% to 60%. Polymerization was carried out with *N*,*N*,*N*′,*N*′′-tetramethylethylenediamine (0.09% vol/vol) and ammonium persulfate (0.04% wt/vol). Electrophoresis for separation of PCR fragments was performed at 90 V and 60°C for 16 h. After electrophoresis, the gels were incubated in ethidium bromide solution (250 ng/ml) for 10 min, rinsed in distilled water, and photographed under UV illumination. Bands representing bacteria were eluted, cloned into the pGEM T-Easy plasmid vector (Promega), and transformed into *Escherichia coli*. For each bacterium, two colonies were randomly picked and sequenced (ABI 3700 DNA analyzer; Macrogen, Inc., Korea), and the results obtained were compared to known sequences by using the BLAST algorithm in the NCBI database.

Screening for the presence of *Rickettsia* sp. Of the three bands detected and analyzed, one showed 98% identity to various *Rickettsia* spp. by BLAST searches. Based on that rickettsial 16S rRNA gene and on similar sequences found in the databases, primers which are capable of specifically amplifying that gene from the whitefly *Rickettsia* sp. were designed. Samples of *B. tabaci* from various

TABLE 2. PCR primer sets used in this study

Gene	Reference	Primer set	Nucleotide sequence (5′ to 3′)
16S rRNA for DGGE	26	341F	CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCCG CCTACGGGAGGCAGCAG
		907R	CCGTCAATTCMTTTGAGTTT
16S rRNA	40	27F	AGAGTTTGATCMTGGCTCAG
		1494R	CTACGGCTACCTTGTTACGA
16S rRNA	This paper	Rb-F	GCTCAGAACGAACGCTATC
		Rb-R	GAAGGAAAGCATCTCTGC
gltA	29	CS1d	ATGACTAATGGCAATAATAA
		CS1273r	CATAACCAGTGTAAAGCTG
		CS409d	CCTATGGCTATTATGCTTGC
	32	RicCS-AR	TGCCCAAGTTCTTTTAACACCTC
ompA	14	70f	ATGGCGAATATTTCTCCAAAA
		701r	GTTCCGTTAATGGCAGCATCT
ompB	13	M59f	CCGCAGGGTTGGTAACTGC
		3599r	TCATTCCGGTTACAGCAAAGT

agricultural crops in Israel were collected in different years and seasons and kept in the laboratory as separate populations as described above or placed in ethanol. To detect the presence of *Rickettsia* in these populations, one adult was ground individually in lysis buffer, as described above, in at least nine replicates. Each sample was subjected to a PCR using the *Rickettsia*-specific primers Rb-F and Rb-R (Table 2), a combination expected to yield a product of about 900 bp. PCR parameters were as follows: denaturation for 2 min at 95°C; 30 cycles of 30 s at 92°C, 30 s at 58°C, and 30 s at 72°C; and a 5-min final extension at 72°C. The whitefly populations screened are summarized in Table 1. All reactions included a negative control of sterile water and a positive control of *Rickettsia*-infected *B. tabaci*. As an internal control, primers known to amplify the mitochondrial cytochrome oxidase I (*COI*) gene (15) were used on all samples.

Characterization of *Rickettsia* sp. in *B. tabaci*. To determine the phylogenetic affiliation of the newly discovered *Rickettsia* sp. with rickettsial groups previously classified (spotted fever, typhus, and ancestral), we have followed the genotypic scheme suggested by Fournier et al. (12). All PCR analyses were performed using specific primers and PCR amplification conditions as specified in the literature (Table 2). A nearly full-length segment of the 16S rRNA gene was obtained using the primer combinations 27F/Rb-R and Rb-F/1494R with the parameters described above. The two 16S rRNA gene contigs were assembled using DNAMAN (Lynnon Biosoft Vaudreuil, Quebec, Canada). The citric acid cycle (citrate synthase) gene *gltA* was amplified and assembled in the same way, and the presence of the rickettsial outer membrane protein (rOmp) encoding genes *ompA* and *ompB* was tested. The sequences of the *Rickettsia* genes obtained were deposited in GenBank.

Establishment of a clean *B. tabaci* line. In order to characterize the *Rickettsia* sp. distribution in *B. tabaci*, a *Rickettsia*-free line that can serve as a negative control was required. Most of the *B. tabaci* populations tested exhibited variation in their infection status (Table 1); therefore, an attempt was made to establish a *Rickettsia*-free line out of the same whitefly colony by isolating 30 mated whitefly females from a line collected in a sweet pepper greenhouse during June 2004 in order to form separate reproductive lines. Each female was allowed to oviposit individually on a sweet pepper (*Capsicum annum*) leaf disk (55-mm diameter) placed on 1% agar in a transparent plastic cup, maintained at 25°C and 60% ± 10% relative humidity and a photoperiod of 14 h of light and 10 h of darkness, until she died. Upon emergence, at least five progeny of each female were placed alive in 96% alcohol, and the infection status of the various lines was tested by subjecting samples from three individuals to PCR with *Rickettsia*-specific primers.

In situ hybridization. Adults, eggs, and the various instars were collected with a needle, while ovaries were dissected in a drop of saline buffer under a stereoscopic microscope. The fluorescence in situ hybridization (FISH) procedure generally followed the method of Sakurai et al. (32), with slight modifications. Specimens were collected directly into Carnoy's fixative (chloroform:ethanol: glacial acetic acid, 6:3:1) and fixed overnight. After fixation, the samples were decolorized in 6% H_2O_2 in ethanol for 2 h and then hybridized overnight in hybridization buffer (20 mM Tris-HCl [pH 8.0], 0.9 M NaCl, 0.01% sodium dodecyl sulfate, 30% formamide) containing 10 pmol of fluorescent probes/ml. Based on the *Rickettsia* sp. and "*Ca*. Portiera aleyrodidarum" 16S rRNA sequences, two DNA probes were designed using Primer3 software (30) (source code available at http://fokker.wi.mit.edu/primer3/) and were checked for specificity using the Ribosomal Database Project II "probe match" analysis tool (http://rdp.cme.msu.edu/); the probe BTP1-Cy3 (5′-Cy3-TGTCAGTGTCAGCC CAGAAG-3′) was designed to specifically target "*Ca*. Portiera aleyrodidarum," and the probe Rb1-Cy5 (5′-Cy5-TCCACGTCGCCGTCTTGC-3′) was designed to target *Rickettsia*. Stained samples were whole mounted and viewed under an IX81Olympus FluoView500 confocal microscope. Specificity of the detection was confirmed using the following controls: no-probe control, RNase-digested control, and *Rickettsia*-free whiteflies.

Nucleotide sequence accession numbers. The sequences of the *Rickettsia* genes obtained in this study were deposited in GenBank under the accession numbers DQ077707 (16S rRNA gene) and DQ077708 (*gltA*).

RESULTS

Identification of bacterial assembly. A banding pattern composed of three distinct bands was evident when the PCR products were analyzed by DGGE (Fig. 1). When these bands were eluted, cloned, sequenced, and compared to other sequences found in GenBank, they showed high similarities to known bacteria; two bands were most similar to the primary whitefly symbiont "*Ca*. Portiera aleyrodidarum" (100%) and the secondary symbiont "*Ca*. Hamiltonella defensa" (100%), respectively. The third band gave a 98% similarity value to the tick symbiont *Rickettsia bellii*. Because the first two bands revealed the presence of previously described bacteria (44), we have concentrated our efforts on characterizing various aspects of the interactions between *B. tabaci* and the newly discovered *Rickettsia* sp.

FIG. 1. DGGE analysis of PCR-amplified, 16S rRNA gene fragments of bacteria found in three individual *Bemisia tabaci* females. *Portiera*, "*Ca*. Portiera aleyrodidarum;" *Hamiltonella*, "*Ca*. Hamiltonella defensa."

Screening for the presence of *Rickettsia* sp. A total of 20 *B. tabaci* populations were screened using the *Rickettsia*-specific primers (Table 1). These populations were collected between the years 1987 and 2003 from Haifa (north) to Hazeva (south), Israel, from eight different host plants (Table 1). *Rickettsia* sp. could be detected in all of the *B. tabaci* populations tested, with infection rates ranging from 22% to 100%.

Establishment of *Rickettsia* sp. identity. The combination of most known *Bacteria* primers with the *Rickettsia*-specific primers Rb-F and Rb-R yielded a 1,445-bp sequence of the 16S rRNA gene which exhibited highest sequence similarity to the proteobacterium *R. bellii* (99%). The use of specific primers for the *gltA* gene resulted in sequences of 1,210 bp showing 97% similarity to the tick symbiont *R. bellii* citrate synthase gene. Presence of the *ompA* and *ompB* genes could not be detected in PCR using specific primers.

Establishment of a clean *B. tabaci* line. Out of 30 isofemale lines, the progeny (F_1) of five females tested negative for *Rickettsia* sp. Consequently, the other 25 lines were discarded, and the five *Rickettsia*-free lines were further reared. The F_2 and F_3 generations of these five lines were also found to be free of *Rickettsia* sp. and were mixed into one population.

In situ hybridization. (i) Primary symbionts. Throughout the life cycle of *B. tabaci*, the probe designed to specifically target the primary symbiont "*Ca*. Portiera aleyrodidarum" consistently produced signal exclusively inside the bacteriomes (Fig. 2 to 4).

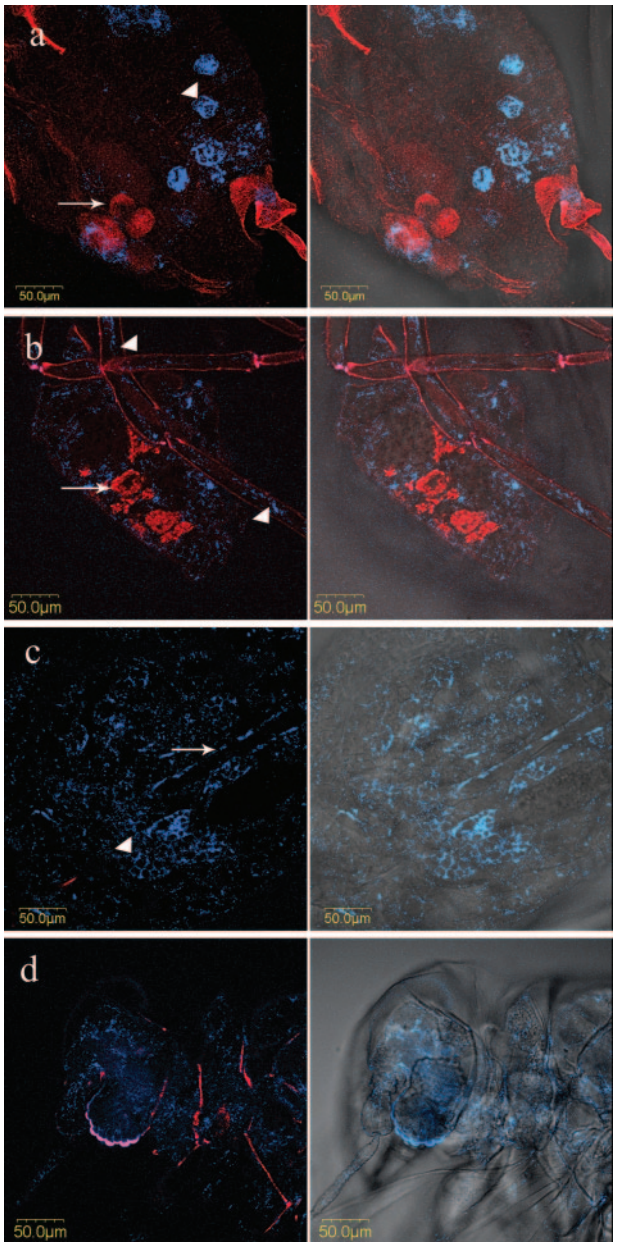

FIG. 2. FISH of *B. tabaci* adults. (a) Bacteriocytes (arrow) and *Rickettsia* sp. (arrowhead) in a female abdomen (combined Z sections). (b) Bacteriocytes (arrow), and *Rickettsia* sp. (arrowhead) in female legs and abdomen (one section). (c) *Rickettsia* sp. concentrated around the follicle cells (arrow head) and the gut tube (arrow) (one section). (d) Female head (one section). Note the chitin autofluorescence. Right panels, bright field and fluorescence; left panels, fluorescence only.

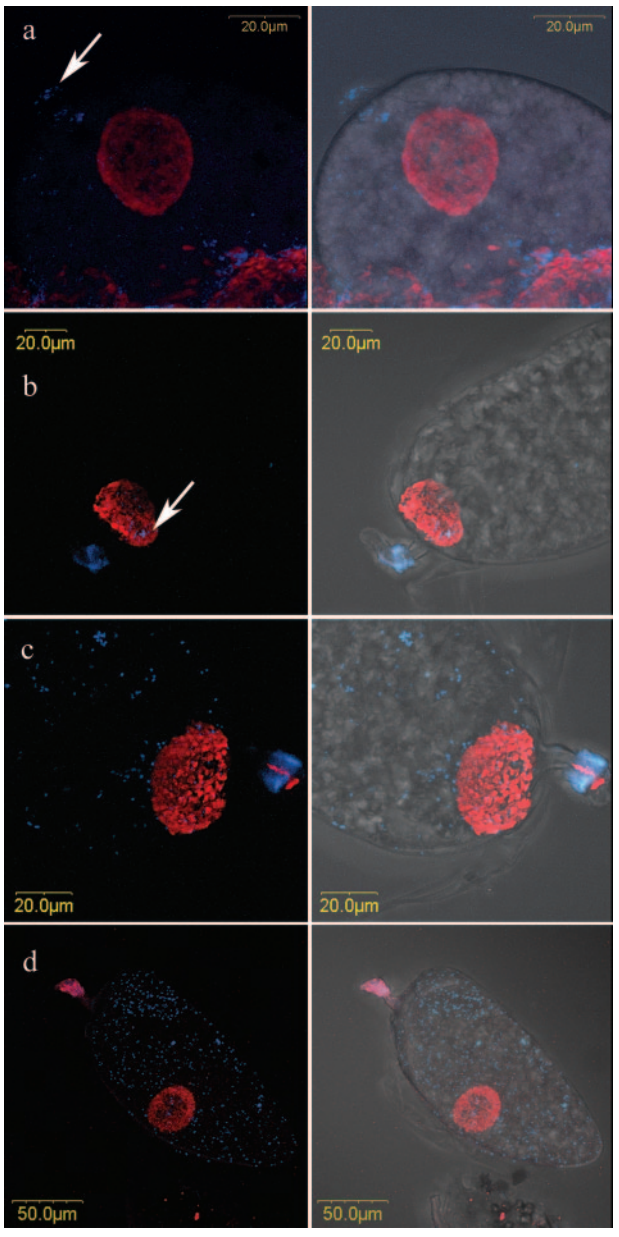

FIG. 3. FISH of *B. tabaci* eggs. (a) Mature oocyte surrounded by external bacteriocytes (red) and a *Rickettsia* sp. (blue). Note the concentration of the *Rickettsia* sp. in the pedicel area (arrow). (b) Less than 24 h old, showing bacteriocyte (red) and *Rickettsia* sp. (blue, arrow). (c) Ca. 48 h old, showing bacteriocyte (red) and *Rickettsia* sp. (blue). (d) More than 48 h old. Note the pedicel autofluorescence. Right panels, combined Z sections of bright field and fluorescence; left panels, fluorescence only.

(ii) Distribution of *Rickettsia* sp. in adults. Spheres of bacteriocytes are seen around and between the ovaries in the female abdomen (Fig. 2a and b). The signal specific to "*Ca*. Portiera aleyrodidarum" indicates that these bacteria are located on the surface and inward of the spheres (Fig. 2b). This organization is in agreement with transmission electron microscopy data that show a large nucleus in the middle of each bacteriocyte (35; M. Ghanim, unpublished data). In females, *Rickettsia* is located around the oocytes, around the follicle cells, and among (but not inside) the bacteriocytes (Fig. 2a to c). In some individuals (males or females), *Rickettsia* is located in specific polygon-like structures (Fig. 2a) and along the gut (Fig. 2c). *Rickettsia* can be detected in the hemolymph of both sexes, occurring in all body parts, including head and legs (Fig. 2b and d). Bacteriocytes are hardly ever detected in males and, overall, *Rickettsia* is more abundant in females (data not shown). Each mature oocyte usually incorporates one bacteriocyte (9, 19) (Fig. 3a), where *Rickettsia* can be hardly de-

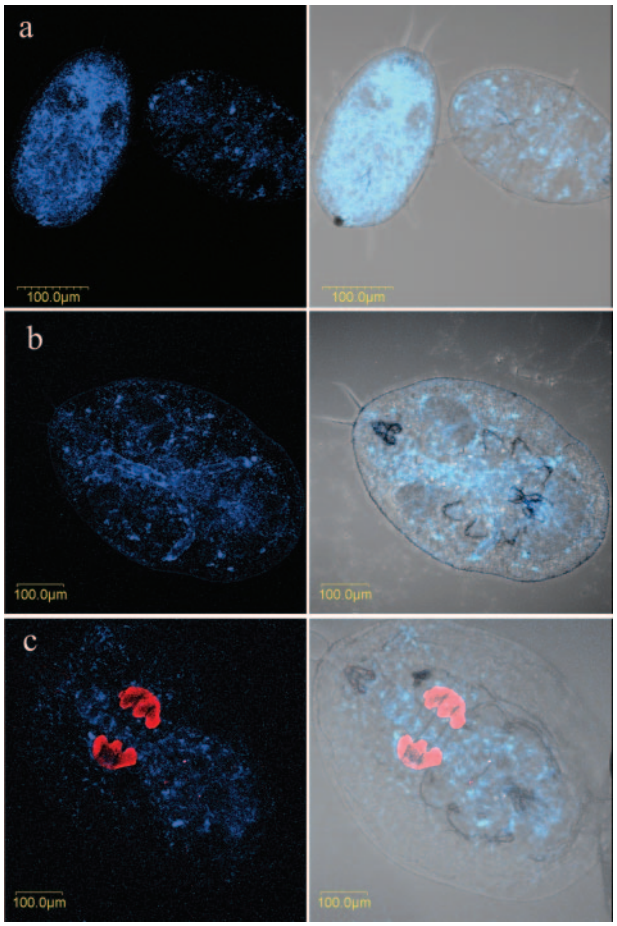

FIG. 4. FISH of *B. tabaci* nymphs. (a) Crawlers; (b) third instar; (c) fourth instar. *Rickettsia* sp. (blue), "*Ca*. Portiera aleyrodidarum" (red). Note the "Y"-shaped distribution. Right panels, combined Z sections of bright field and fluorescence; left panels, fluorescence only.

tected or is detected in the pedicel only (Fig. 3a).

(iii) Distribution of *Rickettsia* sp. in eggs. More than 50 eggs were collected and viewed under the confocal microscope. In embryos 0 to 24 h old, a single bacteriocyte is located near the pedicel. "*Ca*. Portiera aleyrodidarum" is first detected in the bacteriocyte around the perimeter of the cell, with invasion toward the center (Fig. 3b); later on, it occupies most of the bacteriome sphere (Fig. 3c). In older eggs (4 to 5 days of embryogenesis), the bacteriome divides to form two separate organs (data not shown). At early stages of embryogenesis, signal from *Rickettsia* sp. can be seen only among the "*Ca*. Portiera aleyrodidarum" within the bacteriocyte (Fig. 3b). However, at later stages, signal from *Rickettsia* sp. can be detected at a random distribution throughout the embryo (Fig. 3c and d).

(iv) Distribution of *Rickettsia* sp. in nymphs. The distribution of the *Rickettsia* sp. in *B. tabaci* nymphs seems to be random in terms of both quantity and space; the signal in some larvae suggests the presence of large numbers of symbionts, while the signal in others is lower (Fig. 4a). *Rickettsia* seems to be located throughout the nymphal body, excluding the bacteriomes, as in the eggs. Although the signal can be detected throughout the nymph, there is higher intensity in a "Y"-shaped structure, following the contour of the whitefly's gut (19) (Fig. 4b and c). The random spatial distribution can be seen in all nymphal stages (Fig. 4). The absolute confinement of "*Ca*. Portiera aleyrodidarum" to the bacteriomes, as opposed to the characteristic random signal for *Rickettsia* is noticeable when both probes are hybridized on the same crawler (Fig. 4c).

(v) Controls. The no-probe and RNase-digested controls showed fine autofluorescence of the 543 and 633 laser lines. In whiteflies from the *Rickettsia*-free line, bacteriomes are stained with the "*Ca*. Portiera aleyrodidarum"-specific probe, while there is no signal from the *Rickettsia*-specific probe (data not shown).

DISCUSSION

Insects harbor a diverse assemblage of bacteria, and here we applied a method used for assessing microbial compositions in environmental samples for characterizing the microbial assembly in Israeli populations of *B. tabaci*. This community was found to be composed of three different bacteria, namely, "*Ca*. Portiera aleyrodidarum," "*Ca*. Hamiltonella defensa," and *Rickettsia* sp. (Fig. 1). It should be noted, however, that it has been suggested that only bacterial populations that make up 1% of a complex bacterial community can be detected by PCR and DGGE (26) and, in general, PCR amplification with species- or genus-specific primers is more sensitive than broad-range 16S rRNA gene amplifications. The bacteria identified in this study may therefore not represent the entire diversity of the microbial community within that whitefly population, and the presence of other, less numerous bacteria should not be excluded.

The order *Rickettsiales* is composed of a coherent group of obligate intracellular symbionts of eukaryotic cells within the alpha subdivision of the *Proteobacteria* (42). The genus *Rickettsia* is usually described from blood-feeding arthropods, renowned for the ability of some of its members to cause rickettsioses, and was rarely reported from phytophagous insects (1). To establish the identity of the whitefly bacterium and further characterize its phylogenetic affiliation, we followed the guidelines of Fournier et al. (12). These authors suggested that a bacterium can be ascribed the genus *Rickettsia* if it shares >98.1% similarity of the 16S rRNA gene and >86.5% similarity of the *gltA* gene of any known *Rickettsia* sp. The 16S rRNA and *gltA* genes of the symbiont described from *B. tabaci* exhibit 99 and 97% similarity (respectively) with the previously described *Rickettsia bellii*, and that bacterium could therefore be considered a member of the genus *Rickettsia*. According to Fournier et al. (12), a *Rickettsia* sp. in which the *ompA* and *ompB* genes are absent belongs to the ancestral group. Because our PCR analyses failed to detect the presence of these genes, it was concluded that the *B. tabaci Rickettsia* sp. is a member of the ancestral group, together with *R. bellii*. Other than the pea aphid *Rickettsia* sp., the first record of a *Rickettsia* sp. in a phytophagous insect, that bacterium has been reported from the orders Psocoptera, Coleoptera, and Hymenoptera, which are not known to have interaction with vertebrates (20, 22, 41, 43). Outside the Insecta, a *Rickettsia* sp. was also found in

phytophagous organisms, such as the spider mite *Tetranychus urticae* (21).

Whole-mount fluorescence in situ hybridization of various developmental stages established, for the first time, the long-assumed specific localization of the *B. tabaci* primary symbiont "*Ca*. Portiera aleyrodidarum" in the bacteriocytes. This technique also shows a random and uneven localization of the *Rickettsia* sp. in most of the *B. tabaci* eggs, nymphal stages, and adults tested. In various adult and nymph specimens, the bacteria are seen aggregating along the gut structure (Fig. 2 and 4). Although the function of the polygonal structures is currently unknown, this specific *Rickettsia* distribution may serve as a clue in future studies. The detection of *Rickettsia* around the gut structure and in between the follicle cells is unique compared with other localization data: secondary symbionts of plant-feeding insects have been reported from bacteriomes, within the secondary bacteriocytes, and in the hemolymph (aphid) (16, 17) in the syncytium (part of psyllid bacteriome) (34) and within bacteria themselves, forming a secondary symbiosis (in mealybugs) (38). Whole-mount FISH of one *Acyrthosiphon pisum* strain revealed the presence of *Rickettsia* in two types of cells, secondary bacteriocytes and sheath cells. These cells, together with the primary bacteriocytes containing a *Buchnera* sp., form the bacteriome in the body cavity of aphids (32).

The fact that the *Rickettsia* sp. can be detected inside the eggs of *B. tabaci*, and that it is detected throughout the insect development, implies vertical transmission of that bacterium. Our data suggest that the *Rickettsia* enters the oocyte in the ovaries either by penetrating the oocyte via the pedicel (as suggested from Fig. 3) or by penetrating the bacteriocytes. After entering the egg, the bacteria start multiplying and spreading during embryogenesis. The hatching nymph carries *Rickettsia* throughout its body, with seemingly higher concentrations around the gut. Adult females apparently carry a higher load of *Rickettsia* than males, possibly to enable transmission of the bacteria to the next generation.

Studies exploring the influence of secondary symbionts in aphids revealed quite a number of roles these tenants play in their host's biology, including conferring resistance to parasitoids (11, 28), influencing host plant preferences (11, 23, 37), and conferring heat resistance (24). On the other hand, Sakurai et al. (32) showed that a *Rickettsia*-infected *A. pisum* strain exhibits a smaller fresh body weight and a lower total number of offspring than a *Rickettsia*-free strain. These authors also found that the presence of *Rickettsia* significantly suppressed the population of *Buchnera* and postulated that these phenomena may be correlated (32). Other studies investigating the fitness effects of the *Rickettsia* sp. indicate that the presence of that bacterium generally induces negative effects on the aphid hosts, but the intensity of these effects and their consequences depend on environmental factors (6, 7, 24).

The effect of *Rickettsia* sp. on *B. tabaci* is yet to be resolved; however, since it is highly prevalent in all tested Israeli *B. tabaci* populations (Table 1), it can be speculated that its phenotype is advantageous under certain conditions but may be deleterious enough under others to prevent fixation. Moreover, the concentration of the bacteria around the gut tube may indicate a nutritional dependence. Another possible hint for *Rickettsia* influence may come from the work of Gerling and Fried (18) who found a unique phenomenon of density-dependent sterility in the *B. tabaci* parasitoid *Eretmocerus mundus* (Hymenoptera: Aphelinidae) and suggested the involvement of microorganisms. A fitness comparison between *Rickettsia*-infected and noninfected whitefly individuals would provide the information required for understanding the cost and the benefit of this association for the whitefly host.

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