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Isolation and identification of culturable forms of bacteria from the sweet potato whitefly *Bemesia tabaci* Genn. (Homoptera: Aleyrodidae) in Jordan

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Abstract: Homopteran insects contain bacteria in their cells and tissues known as "secondary symbionts," which under special environmental circumstances act against their host insects. In this study, both molecular- and culture-based methods were used to characterize the bacteria associated with the whitefly in Jordan. We isolated, cultured, and identified 11 species of bacteria from nymphs (6 species), adults (8 species), and parasitized pupae (2 species) of the whitefly *Bemesia tabaci* collected from different vegetable crops planted in different localities in Jordan. The identities of the cultured bacteria were evaluated using PCR with sequencing of 16S rRNA gene fragments and fluorescence in situ hybridization. Three gram-negative bacteria were identified as *Erwinia persicinus, Pseudomonas plecoglossicida*, and *Pseudomonas putida*. The identified gram-positive bacteria included *Brevibacterium casei*, *Staphylococcus gallinarum*, *Bacillus pumilus*, *Bacillus licheniformis*, *Bacillus subtilis*, *Exiquobacterium acetylicum*, *Exiguobacterium undae*, *and Micrococcus caseolyticus*.

Key words: Culturable bacteria, PCR, symbiosis, sequencing, *Erwinia persicinus*

Introduction

Members of Hexapoda, the largest class of invertebrates, are involved in several types of symbiosis, primarily with bacteria. All insects live in close association with bacteria; however, bacteria are present on the integument, inhabit the digestive tract, and, in some highly evolved cases, inhabit unique structures within the insect body (Chen et al. 2000; Fukatsu et al. 2000). Many types of bacteria have been identified from different insects, such as the gypsy moth, migratory grasshopper, cabbage moth, and cotton bollworm (Spiteller et al. 2000; Broderick et al. 2004; Xiang et al. 2006). The bacterial association in insects plays a significant role in host insect morphogenesis, food digestion, nutrition, antifungal toxin production, pheromone production, pH regulation, vitamin synthesis, temperature tolerance, resistance against parasitoid development, and detoxification of noxious compounds (Dillon and Dillon 2004; Genta et al. 2006).

Homopteran insects possess a non-culturable intracellular symbiotic bacterium, (*Buchnera* spp.) in the cytoplasm of mycetocytes (or bacteriocytes)—hypertrophied cells in the abdomen specialized for endosymbiosis (Buchner 1965; Baumann et al. 1995). Because homopteran insects live exclusively on

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nutritionally unbalanced food (plant sap) throughout their lives, symbiosis with *Buchnera* is essential for them to compensate for this nutritional deficiency. It has been demonstrated that *Buchnera* symbionts synthesize essential amino acids and other nutrients for their host aphids (Douglas 1989, 1998) and that deprivation of *Buchnera* via antibiotic or heat treatment results in retarded growth, sterility, and/or death in the host insect (Houk and Griffiths 1980; Ohtaka and Ishikawa 1991). Due to their prevalence and importance to homopteran insects, *Buchnera* spp. are often referred to as primary symbionts.

In addition to *Buchnera* primary symbionts, homopteran insects contain additional types of endosymbiotic bacteria, referred to collectively as secondary symbionts or accessory symbionts, which are found in tissues surrounding the bacteriocytes and in specialized secondary bacteriocytes (Buchner 1965; Fukatsu and Ishikawa 1993, 1998; Fukatsu et al. 1998, 2000, 2001; Sandstrom et al. 2001).

The aim of the present study was to culture bacteria generically known as "secondary symbionts" from different stages of the sweet potato whitefly *Bemesia tabaci* that were collected from different host crops. Secondary symbiotic bacteria are the focus of much research, as they may modulate ecologically important traits in homopteran insects, including plant utilization characteristics (Chen et al. 2000; Ferrari et al. 2004; Tsuchida et al. 2004; Wilson et al. 2004), natural enemy resistance (Simon et al. 2003; Ferrari et al. 2004), and thermal tolerance (Chen et al. 2000). Additionally, they could be pathogenic and play a role as biopesticides, such as *Erwinia aphidocola* isolated from the homopteran pea aphid (Harada and Ishikawa 1997). Moreover, the current study represents the first documented identification of symbiotic bacteria in *B. tamaci* using 16S ribosomal DNA (rDNA) sequencing analysis.

Materials and methods

Insect material

The experiment was carried out with the sweet potato whitefly *B. tabaci*, which was collected from different locations in Jordan (Homrat Sahen, Zay, Baqa', Ghor, Irmemeen) from May to December 2006. *B. tabaci* adults and nymphs were collected from cotton, snake cucumber, cauliflower, tomato, and bean plants, and then were surface sterilized with ethanol and household chlorine bleach, as described by Davidson et al. (1994). White fly samples were taken from the surface layer of undisturbed leaves at temperatures that never exceeded 27 °C. Adults and nymphs were processed separately in groups of ca. 100-250 due to their small size. Surface-sterilized insects were homogenized in sterile 0.9% saline. Homogenized insects were plated directly on microbiological media, including nutrient agar, tryptose agar, Luria agar, potato dextrose agar, and blood agar (Sigma, St. Louis, MO, USA), and then were incubated aerobically at 25 °C.

Evaluation of bacterial growth media and growth conditions

Plating samples of melt water on agar-solidified commercial growth media containing low levels of nutrients, such as agar and Difco, or on nutrient agar (Bacto) diluted 100-fold more than normally recommended resulted in the growth of more bacterial colonies than plating on full-strength rich nutrient media. Such nutritionally restricted conditions reduced the tendency of otherwise fast-growing species to dominate during bacterial isolation from environmental samples and apparently also prevented "immediate-growth" stress, which increases the survival and recovery of cells that have accumulated cellular damage during long periods of frozen inactivity. Some bacterial colonies appeared after only 1-2 days of incubation at 37 °C. Nevertheless, upon subsequent sub-culturing, most isolates formed visible colonies after only 2-3 days of incubation at 37 °C. The number of general bacteria counts was determined using the standard determination assay for viable cell counting on a plate the same day as cell collection.

Geographic differences in the number and diversity of bacterial isolates

Most of the isolates had 16S rDNA sequences closely related to non-sporulating gram-positive bacteria or to gram-positive spore-forming bacteria. However, overall, bacteria that belonged to genera were isolated based on 16S rDNA sequences that were 95% identical to that of a previously characterized species. Isolates from both areas (plant spp.) had 16S

rDNA sequences that were >99% identical to the 16S rDNA sequences of the type of bacteria.

Identification of bacterial isolates using 16S rDNA

Total DNA was extracted from bacterial colonies using the method described by Mckee et al. (2003), with minor modifications. The different bacterial colonies isolated from whitefly samples were collected with a sterile inoculation loop and were dispersed in 200 µL of sterile phosphate-buffered saline (PBS). Then the bacterial suspension was centrifuged at 14,000 rpm for 3 min. The collected pellets were re-suspended in 300 µL of CTAB buffer solution and incubated in a water bath at 65 °C for 30 min with continuous shaking, and then bacterial lysates were incubated with 30 µL of 1 mg mL^{-1} of RNase enzyme (Promega, Co., Madison, WI, USA) at 37 °C for 30 min. Next, an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1 v/v/v) was added, mixed, and micro-centrifuged at 14,000 rpm for 5 min. The supernatant was then transferred to a new tube. Afterwards, an equal volume of chloroform/isoamyl alcohol (24:1 v/v) was added, mixed, and micro-centrifuged at 14,000 rpm for 5 min. The supernatant was transferred again to a new tube, 0.6 volume of isopropanol was added and mixed gently, and then incubated at –60 °C for 30 min or overnight at –20 °C until DNA precipitated. Next, the tube was centrifuged at 14,000 rpm for 15 min, the supernatant was discarded, and the precipitated DNA was washed with 1 mL of 70% ethanol, centrifuged at 14,000 rpm for 5 min, and then supernatant was discarded and DNA was dried at room temperature after inverting the tubes on a paper towel to allow all the fluid to dry. Finally, the pellet was re-suspended in 50 µL of TE buffer and stored at –20 °C until used.

The 16S rRNA gene was amplified from bacterial colonies by PCR, using universal eubacterial primer pairs eu27.F and eu1495.R (Weisburg et al. 1991) (Table 1). The PCR reaction mixture (25 µL) included 2 µL of template DNA, 2 U Taq DNA polymerase, 1´ PCR buffer, 1 mM dNTPs mix, 2 mM MgCl2, and 0.2 uM of each primer. PCR was carried out according to the following protocol: 1 cycle at 94 °C for 5 min, 30 cycles at 93 °C for 1 min, 54 °C for 1 min, and 72 °C for 1 min, followed by an extension step at 72 °C for 5 min.

Agarose gel electrophoresis was performed, as described by Sambrook and Russell (2001). Aliquots (10 µL) of each PCR product were resolved electrophoretically on 1%-1.5% agarose gel using 0.5´ TBE buffer. The PCR products were visualized with an UV transilluminator and photographed with a gel documentation system (Gel Doc 200, BIO-RAD, USA) after staining the gel with ethidium bromide (0.5 µg mL^{-1}) (Promega); the DNA molecular weight marker, a 1-kbp DNA ladder (Promega), was used to determine the size of the amplified fragments.

To identify the amplified PCR products we purified them using an EZ-10 Spin Column DNA Cleanup Kit (Bio Basic Inc., Canada) and sequencing at Macrogen, Inc. (Seoul, Korea) using a 3730xl automatic sequencer with BigDye™ terminator cycling conditions. DNA sequencing was performed at Macrogen Inc. on an ANI3730 XL automatic DNA sequencer (www.macrogen.com). The results were then analyzed using the BLAST server at the National Center for Biotechnology Information (NCBI). Alignment analysis was carried out by means of DNAman sequencer analysis software v.5.2.9 (Lynnon BioSoft, Que., Canada) and the sequence for each isolate was compared with GenBank reference sequences using the BLAST system (www.ncbi.com).

Results

In all, 31 bacteria were isolated from *B. tabaci* adults, nymphs, and parasitized pupae (Table 2). The majority of these isolates were gram-positive bacteria (27 isolates). Only 5 of the isolates were cocci-shaped bacteria, while the others were rods or bacilli bacteria. Four isolates were rod-shaped gram-negative bacteria (Table 2).

PCR amplification of the 16S rRNA gene

Nucleic acids extracted from the formed bacterial colonies were subjected to primer pair designation to amplify a region of the 16S rRNA gene and all produced an expected size (ca. 1520 bp), as shown in Figure 1. This result does not indicate that the tested bacteria belong to a specific genus or a particular species, because the primer pair used was a universal eubacterial primer designed to classify related bacteria according to sequencing results.

Table 1. Primer pair used to amplify a portion of the 16S rRNA gene from a different bacterial colony genome.

Primer	Sequence	PCR Amplicon	Reference
eu27.F	5'-GAGAGTTTGATCCTGGCTCAG-3'	1520-bp	Weisburg et al. (1991)
eu1495.R	5'-CTACGGCTACCTTGTTACGA-3'		

Table 2. Designation and description of isolated bacteria from adults, nymphs, and parasitized pupae of *Bemesia tabaci* whitefly.

Bacterial isolates	**Description**
MAZ-1, MAZ-6, MAZ-5, MAZ-7, MAZ-8, MAZ-10, MAZ-11, MAZ-A1, MAZ-B1, MAZ- B4	Gram-positive, catalase-positive, oxidase-negative rods
MAZ-9, MAZ-42	Gram-positive, oxidase-positive, catalase-positive bacilli
MAZ-2, MAZ-4, MAZ-C1, MAZ-C2, MAZ-C3	Gram-positive, oxidase-positive, catalase-positive cocci
MAZ-3	Gram-positive bacilli, positive for catalase and negative for oxidase
MAZ-36, MAZ-37	Gram-negative, oxidase-positive, catalase-positive rods
MAZ-30, MAZ-31, MAZ-32, MAZ-33, MAZ-34, MAZ-35, MAZ-38, MAZ-39, MAZ-B2	Gram-positive, motile, rod-shaped, catalase-positive and oxidase-negative
MAZ-C4	Gram-negative, rod-shaped, motile by one or several polar flagella.
MAZ-40	Gram-negative, oxidase-negative motile, rod-shaped bacterium

Sequencing

The amplified PCR fragments from the different bacteria were purified and sequenced. A sequence similarity search was performed using the BLAST server at NCBI (www.ncbi.nlm.nih. gov/BLAST). The sequences were aligned using DNAMAN software. Sequence analysis showed high genetic similarity and high nucleotide sequence homology. For example, 99% homology was observed between MAZ-30, MAZ-31, MAZ-32, MAZ-33, MAZ34, MAZ-35, MAZ38, and MAZ39 (Table 3). The sequenced results show that a wide range of bacterial types affects the white fly.

Culturable bacteria from *Bemesia tabaci*

Both adults and nymphs of *B. tabaci* harbored culturable forms of bacteria in their cells. Considerable variation in bacterial genera was observed in different individuals. The bacteria identified are summarized in Table 3. In total, 11 different species of bacteria were identified from 7 different genera, from a total of 31 isolates (Table 3) from *B. tabaci* adults and nymphs collected from different vegetable crops in different locations in Jordan.

In all, 8 species of bacteria were isolated from *B. tabaci* adults: *Brevibacterium casei*, *Staphylococcus gallinarum*, *Bacillus pumilus*, *Bacillus licheniformis*, *Bacillus subtilis*, *Exiquobacterium acetylicum*, *Exiguobacterium undae*, and *Pseudomonas plecoglossicida* (Table 4). Six species of bacteria were isolated from *B. tabaci* nymphs: *B. licheniformis, B. pumilus*, *Micrococcus caseolyticus, E. acetylicum*, *Pseudomonas putida*, and *Erwinia persicinus* (Table 5). From parasitized *B. tabaci* pupae on beans collected from the Salt area during August 2006, 2 species of bacteria were isolated (Table 6): *E. acetylicum* and *P. putida* (Table 6).

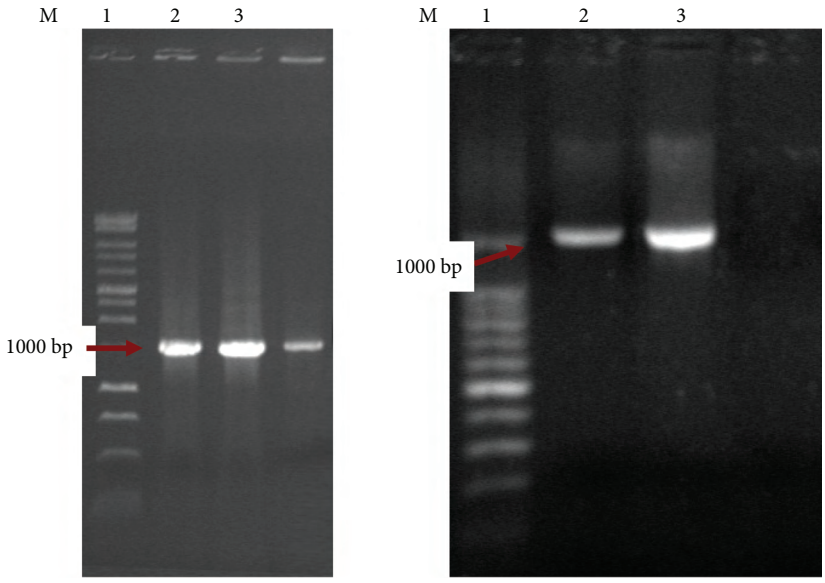

Figure 1. Agarose gel electrophoresis (0.8%) showing PCR products (1520 bp) from 16S rRNA gene of P. larvae. (A): Lanes: Lane M, 1 Kb DNA ladder (the 1500-bp band is indicated); Lane 1, positive control; Lanes 2-3, MAZ- 31 and 32 bacteria. (B): Lane M, 100 bp DNA marker ladder (the 1500-bp band is indicated); Lane 1, positive control; Lane, 2, MAZ-42 bacterium; and Lane 3 being negative control.

Table 3. The 16S rRNA gene sequence of isolated bacteria from whitefly.

Nucleotide identity (%)	**Bacterium**	**Isolate #**
88%	*Bacillus licheniformis*	MAZ-1
99%	*Micrococcus caseolyticus*	MAZ-2
98%	*Brevibacterium casei*	MAZ-3
98%	*Staphylococcus gallinarum*	MAZ-4, MAZ-C2, MAZ-C3
99%	*Bacillus pumilus*	MAZ-5, MAZ-7, MAZ-8, MAZ-10, MAZ-11, MAZ-A1, MAZ-B1, MAZ-B4
98%	*Bacillus licheniformis*	MAZ-6
99%	*Bacillus subtilis*	MAZ-9
98%-99%	*Exiquobacterium acetylicum*	MAZ-30, MAZ-31, MAZ-32, MAZ-33, MAZ-34, MAZ-35, MAZ-38, MAZ-39
97%	*Pseudomonas putida*	MAZ-36, MAZ-37
100%	*Bacillus subtilis*	MAZ-42
99%	*Erwinia persicinus*	MAZ-40
99%	*Exiguobacterium undae*	MAZ-B2
95%	*Pseudomonas plecoglossicida*	MAZ-C4
99%	*Staphylococcus gallinarum*	MAZ-C1

Table 4. Designation, identified name, host and location of isolated bacteria from adults of *Bemesia tabaci* whitefly.

Designation	Identified name	Host	Location
MAZ-3	*Brevibacterium casei*	Cotton	Homrat Sahen
MAZ-4 MAZ-C1 MAZ-C2 MAZ-C3	*Staphylococcus gallinarum*	Cotton	Homrat Sahen
MAZ-6	*Bacillus licheniformis*	Cotton	Homrat Sahen
MAZ-9	*Bacillus subtilis*	Snake-Cucumber	Baqa'
MAZ-11	*Bacillus pumilus*	Snake-Cucumber	Zay
MAZ-33	*Exiquobacterium acetylicum*	Snake-Cucumber	Zay
MAZ-4	*Bacillus subtilis*	Cauliflower	Baqa'
MAZ-A1 MAZ-B1 MAZ-B4	*Bacillus pumilus*	Cotton	Homrat Sahen
MAZ-B2	*Exiguobacterium undae*	Cotton	Homrat Sahen
MAZ-C4	*Pseudomonas plecoglossicida*	Cotton	Homrat Sahen

Table 5. Designation, identified name, host and location of isolated bacteria from nymphs of *Bemesia tabaci* whitefly.

Designation	Identified name	Host	Location
Maz-1	*Bacillus licheniformis*	Cotton	Homrat Sahen
Maz-2	*Micrococcus caseolyticus*	Snake-cucumber	Salt
MAZ-7, MAZ-8	*Bacillus pumilus*	Snake-cucumber	Salt
MAZ-10	*Bacillus pumilus*	Cotton	Homrat Sahen
MAZ-30	*Exiquobacterium acetylicum*	Cucumber	Salt
MAZ-31	*Exiquobacterium acetylicum*	Tomato	Zay
MAZ-32	*Exiquobacterium acetylicum*	Snake-cucumber	Salt
MAZ-35	*Exiquobacterium acetylicum*	Snake-cucumber	Zay
MAZ-36	*Pseudomonas putida*	Snake-cucumber	Salt
MAZ-38	*Exiquobacterium acetylicum*	Tomato	Irmemeen
MAZ-40	*Erwinia persicinus*	Cauliflower	Ghor

Table 6. Designation, host and location of isolated bacteria from parasitized pupae of *Bemesia tabaci* whitefly.

Designation	Identified name	Host	Location
MAZ-34	*Exiquobacterium acetylicum*	Beans	Salt
MAZ-37	*Pseudomonas putida*	Beans	Salt

Discussion

Almost all homopteran insects have primary symbiotic bacteria of the genus *Buchnera* (Munson et al. 1991), which are required for normal growth and reproduction. In addition to *Buchnera*, many homopteran insect species house symbionts that are not found in all host individuals; therefore, they are considered secondary symbionts. Nevertheless, these symbionts may occur at high frequencies in host populations (Chen and Purcell 1997; Tsuchida et al. 2002; Leonardo and Muiru 2003; Simon et al. 2003); however, most research on symbiosis in homopteran insects was performed with one species of aphids: the pea aphid *Acyrthosiphon pisum* (Harris). One of the best-studied insect symbioses is that of the pea aphid and its obligate bacterial symbiont, *Buchnera*. *Buchnera* was reported to synthesize essential amino acids from precursors in the nutrient-poor plant sap on which the aphid feeds (Douglas and Prosser 1992; Lai et al. 1994; Baumann et al. 1995). The pea aphid has become a model system for studying facultative symbiosis, in part because it houses a diversity of facultative bacterial symbionts. To date, 5 S-symbionts have been reported from *A. pisum*: PASS (after *p*ea *a*phid *s*econdary *s*ymbiont) (Chen and Purcell 1997; Chen et al. 2000), R-type symbiont (Sandstrom et al. 2001) or simple S-symbiont (Unterman et al. 1989; Fukatsu et al. 2000), PABS (after *p*ea *a*phid *Bemisia*-type *s*ymbiont) (Darby et al. 2001) or T-type symbiont (Sandstrom et al. 2001), *Rickettsia* symbiont (Chen et al. 2000) or PAR (after *pea* aphid *Rickettsia*) (Chen et al. 1996, 2000), and PAUS (after *p*ea *a*phid *U*-type *s*ymbiont) or U-type symbiont (Sandstrom et al. 2001).

The inability, as yet, to culture the primary *Buchnera* intracellular symbionts makes it difficult to study the possible nutritional or other functions of these bacteria (Chen et al. 2000). To date, the role of the primary endosymbiont *Buchnera* in host aphids has been deduced mainly from studies of aposymbiosis, i.e. by eliminating endosymbionts from aphids using heat treatment (Ohtaka and Ishikawa 1991) or antibiotics (Prosser and Douglas 1991), and by the homology of symbiont genes to known bacterial genes, such as those coding for enzymes involved in tryptophan synthesis (Munson and Baumann 1993) or chaperonins (Ohtaka et al. 1992), among many others. For the previously mentioned reasons, the present study focused on the secondary symbionts that can be cultured, enabling us to study their insecticidal activity (Ateyyat et al. 2010).

Previous reports have described the isolation of bacteria from different insects (Spiteller et al. 2000; Broderick et al. 2004; Xiang et al. 2006). However, most of these are reports of single-sampling experiments, whereas we compared the numbers and types of bacteria that were recovered from *B. tabaci* from different plant species at different locations. The present study is the first to identify bacteria associated with the whitefly *B. tabaci* in a comprehensive manner, using 16S ribosomal DNA (rDNA) sequencing analysis.

There is a need for better understanding of the diversity of whitefly-associated bacteria using molecular techniques. Such techniques are very relevant to the isolation of novel strains with desirable functional characteristics and biotechnological applications. These techniques were used previously to study the bacterial community in aphids (Chen et al. 2000; Fukatsu et al. 2000). New genome sequences can be used to infer phylogenetic relationships between prokaryotes, which deal with the organization and evolution of microbial genomes, mechanisms of transmission, and the exchange and reshuffling of genetic information; However, the choice of microbes to be sequenced seems to follow an individualistic trend in which choices are made according to the institution and the environment in which it is situated.

Phenotypic techniques are generally used to characterize bacterial strains associated with insects. Davidson et al. (2000) isolated and cultured bacteria associated with another type of whitefly, the silverleaf whitefly *Bemesia argentifolii*, which is one of the most damaging insects to agriculture in the southern United States. They used microscopic appearance, Gram staining, anaerobic growth, catalase production, colony morphology, and reactions on API identification strips to identify the spore-forming aerobic bacteria *B. licheniformis* (Weigman), *B. megaterium* deBary, *B. amyloliquefasciens* Fukumoto, and *B. subtilis* (Ehrenberg). Moreover, on the basis of API tests, Davidson et al. (2000) identified short gram-negative or gram-variable pleomorphic rods as *Enterobacter cloacae* (Jordan), *Flavimonas oryzihabitans* Holmes et al., *Citrobacter* sp. Workman and Gillen, *Cellulomonas* sp. Bergey et al., *Chryseomonas luteola* Holmes et al., *Acinetobacter lwoffsii* Brisou & Prevot, and *A. baumanni* (Deacon). The problem with such a technique is that some strains of bacteria, particularly gram-positive, produced no reaction on API tests and therefore remain unidentified (Davidson et al. 2000; Ateyyat 2008). Gas chromatography (GC) fatty acid profiles using the MIDI identification system is another technique used for identifying bacteria associated with insects (Davidson et al. 2000; Ateyyat 2008). Only bacteria that can be cultured on soy tryptic agar media (TSA) and incubated at temperatures lower than 28 °C for 24 h could be identified using this technique (Ateyyat 2008).

In the present study 11 different species of bacteria from 7 genera were identified using molecular biology tools from a total of 31 isolates. These bacteria were isolated from *B. tabaci* adults, nymphs, and parasitized pupae. In all, 87% of these isolates were gram-positive bacteria, of which about 84% were rod-shaped bacteria. Variation was observed in the isolates based on crop species and location. More than 50% of the isolates were identified as *B. pumilus* and *E. acetylicum*. *Exiquobacterium acetylicum* was isolated from *B. tabaci* adults, nymphs, and parasitized pupae collected from different crops at different locations. *Pseudomonas putida* was collected from 2 different crops at the same location. *Bacillus licheniformis* was isolated only from whitefly collected from cotton plants. *Micrococcus caseolyticus* was isolated only from whitefly nymphs collected from snake-cucumber. *Erwinia persicinus* was isolated only from whitefly nymphs collected from cauliflower. *Exiguobacterium undae* was isolated only from whitefly adults collected from cotton. *Bacillus subtilis* was isolated only from whiteflies collected from 2 different crops planted at the same location. *Brevibacterium casei* was isolated only from whiteflies collected from 1 location. Whiteflies collected from cotton plants harbored the majority of bacterial species (6 species).

In the present study we identified only culturable forms of bacteria that are known as secondary symbionts or opportunistic bacteria. Some of these bacteria originally resided in plant tissues, mainly inside vascular tissues, without doing harm to the plant, and then transferred to the whiteflies through probing sap from the vascular tissues of their host plants (Fukui et al. 1999a, 1999b; Kobayashi and Palumbo 2000). *Erwinia persicinus* was isolated from *B. tabaci* collected from cauliflower in Jordan. This bacterial species was isolated by Hao et al. (1990) from tomato plants.

Microorganisms that can grow on the phyllosphere may be better candidate BCAs than those that cannot (Andrews, 1992). Consequently, culturable whitefly-associated bacteria may be effective as BCAs against *B. tabaci*. For this purpose, the insecticidal activity of the 11 identified bacterial strains against the sweet potato whitefly was evaluated against *B. tabaci* 2nd nymphal instars. The observed mortality of *B. tabaci* 2nd nymphal instars exceeded 50% using *Erwinia percisinus*, which was isolated from whiteflies collected from cauliflower plants (Ateyyat et al. 2010).

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References

Andrews JH (1992) Biological control in the phyllosphere. Annu Rev Phytopathol 30: 603-635.

Ateyyat M. (2008) Culturable bacteria associated with the guts of pea aphid, *Acyrthosiphum pisum* (Homoptera: Aphididae). J Entomol 5: 167-175.

Ateyyat MA, Shatnawi M, Al-Mazra'awi MS (2010). Culturable Whitefly-associated Bacteria and their Potential as Biological Control Agents. Jordan J Biol Sci (accepted).

Baumann P, Baumann L, Lai CY, Rouhbakhsh D, Moran NA, Clark MA (1995) Genetics, physiology and evolutionary relationships of the genus *Buchnera*: intracellular symbionts of aphids. Ann Rev Microbiol 49: 55-94.

Broderick NA, Raffa KF, Goodman RM, Handelsman J (2004) Census of the bacterial community of the gypsy moth larval midgut by using culturing and culture independent methods. Appl Environ Microbiol 70: 293-300.

Buchner P (1965) Endosymbiosis of Animals with Plant Microorganisms. Interscience, New York.

Chen DQ, Montllor CB, Purcell AH (2000) Fitness effects of two facultative endosymbiotic bacteria on the pea aphid, *Acyrthosiphon pisum*, and the blue alfalfa aphid, *A. kondoi*. Ent Exp et App 95: 315-323.

Chen DQ, Purcell AH (1997) Occurrence and transmission of facultative endosymbionts in aphids. Current Microbiol 34: 220-225.

Chen DQ, Campbell BC, Purcell AH (1996) A new rickettsia from a herbivorous insect, the pea aphid *Acyrthosiphon pisum* (Harris). Current Microbiol 33: 123-128.

Darby AC, Birkle LM, Turner SL, Douglas AE (2001) An aphid-borne bacterium allied to the secondary symbionts of whitefly. FEMS Microbiol Ecol 36: 43-50.

Davidson EW, Rosell RC, Hend DR (2000) Culturable Bacteria associated with the whitefly, *Bemesia Argentifolii* (Homoptera: Aleyrodidae). Fl Ent 83: 159-171.

Davidson EW, Segura BJ, Steele T, Hendrix DL (1994) Microorganisms influence the composition of honeydew produced by the silverleaf whitefly, *Bemisia argentifolii*. J Insect Physiol 40: 1069-1076.

Dillon RJ, Dillon VM (2004) The gut bacteria of insects: Nonpathogenic interactions. Annu Rev Entomol 49: 71-92.

Douglas AE (1989) Mycetocyte symbiosis in insects. Biol Rev 64: 409-434.

Douglas AE (1998) Nutritional interactions in insect-microbial symbioses: aphids and their symbiotic bacteria *Buchnera*. Ann Rev Entomol 43: 17-37.

Douglas AE, Prosser WA (1992) Synthesis of the essential amino acid tryptophan in the pea aphid (*Acyrthosiphon pisum*) symbiosis. J Insect Physiol 33: 565-568.

Ferrari J, Darby AC, Daniell TJ, Godfray HCJ, Douglas AE (2004). Linking the bacterial community in pea aphids with host-plant use and natural enemy resistance. Ecol Entomol 29: 60-65.

Fukatsu T, Ishikawa H (1993) Occurrence of chaperonin 60 and chaperonin 10 in primary and secondary bacterial symbionts of aphids: implications for the evolution of an endosymbiotic system in aphids. J Mol Evol 36: 568-577.

Fukatsu T, Ishikawa H (1998) Differential immunohistochemical visualization of the primary and secondary intracellular symbiotic bacteria of aphids. Appl Entoml Zool 33: 321-326.

Fukatsu T, Nikoh N (1998) Two intracellular symbiotic bacteria from the mulberry psyllid *Anomoneura mori* (Insecta, Homoptera). Appl Environ Microbiol 64: 3599-3606.

Fukatsu T, Nikoh N, Kawal R, Koga R (2000) The secondary endosymbiotic bacterium of the pea aphid *Acyrthosiphon pisum* (Insecta: Homoptera). Appl and Environ Microbiol 66: 2748-2758.

Fukatsu T, Tsuchida T, Nikoh N, Kawal R, Koga R (2001) Spiroplasma symbiont of the Pea Aphid *Acyrthosiphon pisum* (Insecta: Homoptera). Appl and Environ Microbiol 67: 1284-1291.

Fukui R, Fukui H, Alvarez AM (1999a) Comparisons of single versus multiple bacterial species on biological control of anthurium blight. Phytopathol 89: 366-373.

Fukui R, Fukui H, Alvarez AM (1999b) Suppression of bacterial blight by a bacterial community isolated from the guttation fluids of anthuriums. Appl Environ Microbiol 65: 1020-1028.

Genta FA, Dillon RJ, Terra WR, Ferreira C (2006) Potential role for gut microbiota in cell wall digestion and glucoside detoxification in *Tenebrio molitor* larvae. J Insect Physiol 52: 593-601.

Hao MV, Brenner DJ, Steigerwalt AG, Kosako Y, Komagata K (1990) *Erwinia persicinus*, a new species isolated from plants. Int J Syst Bacteriol 40: 379-83.

Harada H, Ishikawa H (1997) Experimental pathogenicity of *Erwinia aphidocola* to pea aphid, *Acyrthosiphon pisum*. J Gen Appl Microbiol 43: 363-367.

Houk EJ, Griffiths GW (1980) Intracellular symbiotes of the Homoptera. Ann Rev Entomol 25: 161-187.

Kobayashi DY, Palumbo JD (2000) Bacterial endophytes and their effects on plants and uses in agriculture. In: Bacon CW, White JF, Jr (Eds.) Microbial Endophytes, Marcel Dekker, New York.

Lai CY, Baumann L, Baumann P (1994) Amplification of *trpEG*: adaptation of *Buchnera aphidicola* to an endosymbiotic association with aphids. Proc Nat Acad Sci, USA 91: 3819-3823.

Leonardo TE, Muiru GT (2003) Facultative symbionts are associated with host plant specialization in pea aphid populations. Proc R Soc Lond B (Suppl.): 270, S209-S212.

Mckee D, Atkinson SE, Collings, Eaton JW, Gill AB, Harvey I, Hatton K, Heyes T, Wilson D, Moss B (2003) Response of freshwater microcosm communities to nutrients, fish, and elevated temperature during winter and summer. Limnol Oceanogr 48: 707-722.

Munson MA, Baumann P (1993) Molecular cloning and nucleotide sequence of a putative Trpdc(F)Ba operon. J Bacteriol 175: 6426-6432.

Munson MA, Baumann P, Clark MA, Baumann L, Moran NA, Voegtlin DJ (1991) Evidence for the establishment of aphid-eubacterium endosymbioses in an ancestor of four aphid families. J Bacteriol 173: 6321-6324.

Ohtaka C, Ishikawa H (1991) Effects of heat treatment on the symbiotic system of an aphid mycetocyte. Symbiosis 11: 19-30.

Ohtaka C, Nakamura H, Ishikawa H (1992) Structures of chaperonins from an intracellular symbiont and their functional expression in *Escherichia coli* groE mutants. J Bacteriol 174: 1869-1874

Prosser WA, Douglas AE (1991) The aposymbiotic aphid: an analysis of chlortetracycline-treated pea aphid *Acyrthosiphon pisum*. J Insect Physiol 37: 713-719.

Sambrook J, Russell DW (2001) Molecular Cloning: A Laboratory Manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Sandstrom JP, Russell JA, White JP, Moran NA (2001) Independent origins and horizontal transfer of bacterial symbionts of aphids. Mol Ecol 10: 217-228.

Simon JC, Carre S, Boutin M, Prunier-Leterme N, Sabater- Munoz B, Latorre A. (2003) Host-based divergence in populations of the pea aphid: insights from nuclear markers and the prevalence of facultative symbionts. Proc R Soc Lond B 270: 1703-1712.

Spiteller D, Dettner K, Boland W (2000) Gut bacteria may be involved in interactions between plants, herbivores and their predators: microbial biosynthesis of N-acylgluta-mine surfactants as elicitors of plant volatiles. Biol Chem 381: 755-762.

Tsuchida T, Koga, R., Fukatsu T (2004) Host plant specialization governed by facultative symbiont. Science: 303, 1989.

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*. Mol Ecol 11: 2123-2135.

Unterman BM, Baumann P, McLean DL (1989) Pea aphid symbiont relationships established by analysis of 16S rRNAs. J Bacteriol 171: 2970-2974.

Weisburg W, Barns S, Pelletier D, Lane D (19991) 16S Ribosomal DNA amplification for phylogrnrtic Study. J Bacterial 173: 697-70.

Wilson ACC, Massonnet B, Simon JC, Prunier-Leterme N, Dolatti L, Llewellyn KS, Figueroa CC, Ramirez CC, Blackman RL, Estoup A, Sunnucks P (2004) Cross-species amplification of microsatellite loci in aphids: assessment and application. Mol Ecol Notes 4: 104-109.

Xiang H, Wei GH, Jia S, Huang J, Miao XX, Zhou Z, Zhao LP, Huang YP (2006) Microbial communities in the larval midgut of laboratory and field populations of cotton bollworm (*Helicoverpa armigera*). Can J Microbiol 52: 1085-1092.