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Culturable Whitefly Associated Bacteria and Their Potential as Biological Control Agents

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Abstract

Bio-pesticides play an important role in reducing the deleterious effects associated with using conventional insecticides. For this reason, the potential of eleven whitefly-associated bacterial isolates as biological control agents was studied under lab conditions. These bacteria were three gram negatives; *Erwinia persicinus, Pseudomonas plecoglossicida* and *Pseudomonas putida*, and 8 gram positives; *Brevibacterium casei*, *Staphylococcus gallinarum*, *Bacillus pumilus*, *Bacillus licheniformis*, *Bacillus subtilis*, *Exiquobacterium acetylicum*, *Exiguobacterium undae*, and *Micrococcus caseolyticus*. Results revealed that *Erwinia persicinus, Bacillus pumilus* and *Exiquobacterium acetylicum* were the most effective in reducing *Bemisia tabaci* 2nd nymphal instar populations. *Erwinia persicinus* was the most promising bacterial isolate to be developed as a biological control agent

الملخص

يلعب استخدام المبيدات الميكروبية دورا هاما في التقليل من الآثار الضارة المصاحبة لاستخدام المبيدات الحشرية التقليدية. لذلك فقد كان الهدف من هذا البحث هو تجربة إمكانية استخدام إحدى عشرة عزلة بكتيرية تم عزلها من جسم حشرة الذبابة البيضاء بهدف استخدامها كمبيدات بكتيرية على نفس الحشرة التي عزلت منها. جربت ثلاث عزلات بكتيرية سالبة لصبغة غرام هي Erwinia persicinus، Pseudomonas plecoglossicida و Pseudomonas putida و ثماني عزلات موجبة لصبغة غرام هي:
Brevibacterium casei, Staphylococcus gallinarum, Bacillus pumilus, Bacillus licheniformis, Bacillus subtilis, Exiquobacterium acetylicum, Exiguobacterium undae, و Micrococcus caseolyticus.
أظهرت النتائج بأن العزلات البكتيرية Erwinia persicinus، Bacillus pumilus و Exiquobacterium acetylicum كانت الأكثر تأثيرا في تخفيض أعداد العمر اليرقي الثاني لحشرة الذبابة البيضاء. هذا واعطت العزلة البكتيرية Erwinia persicinus نتائج واعدة وتبشر بإمكانية استخدامها كمبيد حيوي.

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Keywords: Bio-insecticide, IPM, Mortality, Aleurodidae, *Bemisia tabaci, Erwinia persicinus.*

1. Introduction

The sweet potato whitefly, *Bemisia tabaci* Gen. (Homoptera: Aleurodidae), is a key pest of vegetables in Jordan (Al-Musa et al., 1987). It is also a serious polyphagous economic pest attacking more than 600 plant species worldwide of agronomic, horticultural, and ornamental crops (Gennadius, 1989; Byrne et al., 1990; Brown, 1994). Whitefly management has not traditionally relied on neonicotinoid use (McKenzie et al., 2005), it currently relies on these pesticides. The adverse effects are likely, regardless of the chemical used, and are not particular trait of neonicotinoids (Palumbo et al., 2001). However, the increasing resistance of *Bemisia* species to insecticides provides an impetus to use integrated pest control measures, including biopesticides and biological control to combat this pest (Ateyyat, 2009b). To date, no bacterial insecticide has been discovered with sufficient activity against whiteflies to warrant commercial production. Insect-associated bacteria may be promising to control whiteflies. For example *Enterobacter cloacae* exhibited a mild pathogenicity with 34% mortality towards the silver leaf whitefly, *Bemisia argentifolii* (Davison et al., 2000). Thus, the present study is attempted to investigate the toxic potential of eleven whitefly-associated bacterial isolates that were isolated from adults and nymphs of *B. tabaci* collected from different host plants grown in different regions of Jordan in 2007 (Ateyyat et al., 2009a) towards the sweet potato whitefly, *B. tabaci*.

2. Materials and Methods

2.1. Bacterial Isolates

The tested bacteria were three gram negatives; Erwinia persicinus,Pseudomonas plecoglossicida and Pseudomonas putida, and 8 gram positives; Brevibacterium casei, Staphylococcus gallinarum, Bacillus pumilus, Bacillus licheniformis, Bacillus subtilis, Exiquobacterium acetylicum, Exiguobacterium undae, and Micrococcus caseolyticus. Designation, bacterium name and source of isolated bacteria from Bemisia tabaci whitefly are presented in Table 1.

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Table 1. Designation, bacterium name, and source of isolated bacteria from *Bemisia tabaci* whitefly.

Designation	*Bacterium*	Source		
		B. tabaci stage	Host plant	Location
MAZ-1	*Bacillus licheniformis*	nymphs	Cotton	Homrat Sahen
MAZ-2	*Micrococcus caseolyticus*	nymphs	Snake-Cucumber	Salt
MAZ-3	*Brevibacterium casei*	adults	Cotton	Homrat Sahen
MAZ-4	*Staphylococcus gallinarum*	adults	Cotton	Homrat Sahen
MAZ-5	*Bacillus pumilus*	nymphs	Snake-Cucumber	Salt
MAZ-9	*Bacillus subtilis*	adults	Snake-Cucumber	Baqa'
MAZ-30	*Exiquobacterium acetylicum*	nymphs	Cucumber	Salt
MAZ-36	*Pseudomonas putida*	nymphs	Snake-Cucumber	Salt
MAZ-40	*Erwinia persicinus*	nymphs	Cauliflower	Ghor
MAZ-B2	*Exiguobacterium undae*	adults	Cotton	Homrat Sahen
MAZ-C4	*Pseudomonas plecoglossicida*	adults	Cotton	Homrat Sahen

2.2. Enrichment of Bacterial Isolates

The bacterial isolates were enriched by inoculation into 100 ml of nutrient broth (Oxoid Ltd, Cambridge, UK); amended with 0.1% Tween 20; and incubated in an orbital shaker incubator (Orbital 4535**,** Farma Scientific, Canada) at 28°C with shaking at 150 rpm for overnight. After incubation, bacterial culture broths were adjusted at 600 nm to get an optical density of (0.5) where $A_{600nm}^{=0.5}$ was equivalent to 1 x 10^8 CFU/ml.

2.3. Bemisia Tabaci Culture

Bemisia tabaci was cultured in a controlled greenhouse compartment at 24 ± 2°C and a 16h photoperiod on potted cotton plants, *Gossypium hirsutum* L. The plants were grown in 15 cm diameter pots filled with a mixture of 1:1 sand and peat moss. To obtain *B. tabaci* 2nd instars of uniform ages, tomato plants (variety Guardian, Enza Zaden, Jordan) with 4–5 leaves were exposed to oviposition by placing within the infested cotton plants in the culture. After a 24–48h oviposition period, plants were removed from the culture and all adult *B. tabaci* were aspirated from them. The plants were then placed in a *B. tabaci* free greenhouse compartment at 24 ± 2°C and a 16h photoperiod where *B. tabaci* eggs were allowed to hatch and the nymphs develop for 9–10 days to second instar nymphs.

2.4. Mortality effects on B. tabaci 2nd instar nymphs

The effect of the bacterial isolates on the sweet potato whitefly was studied under laboratory conditions using a leaf-dip bioassay. A leaf cage was prepared from two 9 cm Petri plates by attaching the bottom of the upper plate to the cover of the lower plate. A four mm hole was made through the two plates. Tomato leaflets infested with 2nd instar nymphs of the sweet potato whitefly (as described above) were inserted through those holes following treatment. Tomato leaflets were treated by immersion in each isolate broth for 5 s. The treated leaflets were placed under a laminar hood until air dried after they were transferred to the leaf cages. A two cm opening covered with whitefly proof muslin was cut in the cover of the upper plate. The bottom of the lower plate was filled with water to prevent the wilting of the tomato leaflet. A 9 cm filter paper was placed in the bottom of the upper plate to provide additional humidity. Each leaf cage was assigned randomly to one of the isolates and to a control treatment. The control treatment used non-inoculated broth. There were 7 replicates (leaf cages) for each isolate. The leaf cages were incubated at 24°C ±2 and a 16h photoperiod, and whitefly mortality was recorded on 1, 3, and 5d post application. A whitefly nymph was considered dead if it was shriveled or if its color changed to brown when compared to the normal pale yellow color. In order to confirm pathogenicity of bacteria showed toxic activity against whiteflies, these bacterial isolates were recovered from the dead inoculated whiteflies with these bacterial isolates. The liquid culture of these bacterial isolates showing toxic activity against the whiteflies was centrifuged into the supernatant and pellet. If the bioassay with the supernatant caused high mortality, then the bacteria is considered toxic to whiteflies.

2.5. Effects of bacterial concentration on B. tabaci 2nd instar nymphs.

Based on the results from the previous bioassay, three bacterial isolates *Erwinia persicinus, Bacillus pumilus*, and *Exiquobacterium acetylicum* were selected for further studies. Broths of these bacterial isolates were enriched as described above and measured by a spectrophotometer at 600 nm to get three different optical densities (0.5, 1, and 2) where $A_{600nm}{}^{=1}$ was equivalent to 2×10^9 CFU/ml. The bacterial isolate and density combinations were bio-assayed as above except that mortality was recorded 5d post application.

2.6. Statistical Analysis.

Arcsine-transformed percentage data were subjected to a one-way ANOVA, followed by a Least Significant Differences test at 95% confidence level (SAS Institute, 2005).

3. Results

Bemisia tabaci 2nd nymphal instars showed no significant changes in the shape and color when treated with the non-inoculated broth. Twenty four hours post treatment, all tested bacterial isolates, produced significantly (*F =262.15; df=11,72; P<0.001*) greater mortality towards *B. tabaci* 2nd nymphal instars when compared with the control treatment (Fig.1).

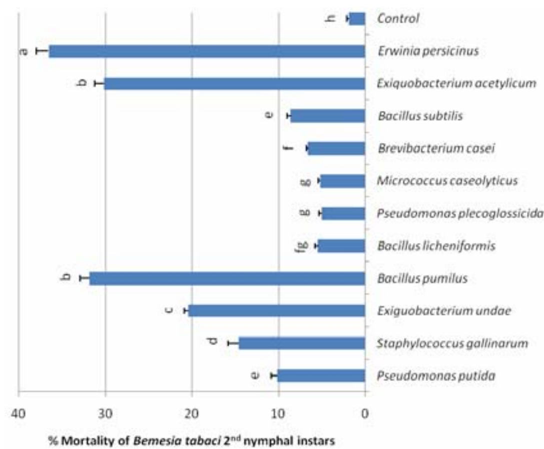

Figure 1.Percentage mortality of *Bemisia tabaci* 2nd nymphal instars (± SE), 1 day post application with bacteria. Percentages were arcsine transformed before analysis. Means with the same letter are not significantly different using LSD at 95 % confidence level.

Pathogenicity of these bacteria was confirmed as these bacteria were recovered from the dead inoculated whiteflies with these bacterial isolates. *Erwinia persicinus, Bacillus pumilus*, and *Exiquobacterium acetylicum* gave levels of insect mortality that was significantly greater than those produced by the other bacterial isolates over the same period (Fig. 1). After 3 days, *Erwinia persicinus, Bacillus pumilus*, and *Exiquobacterium acetylicum* continued to produce significantly higher levels of mortality (*F =134.17; df.=11,72; P<0.001*) towards the *B. tabaci* 2nd nymphal instars in comparison with the other bacterial isolates (Fig. 2). Even though an increase in the mortality of *B. tabaci* 2nd nymphal instars after using the

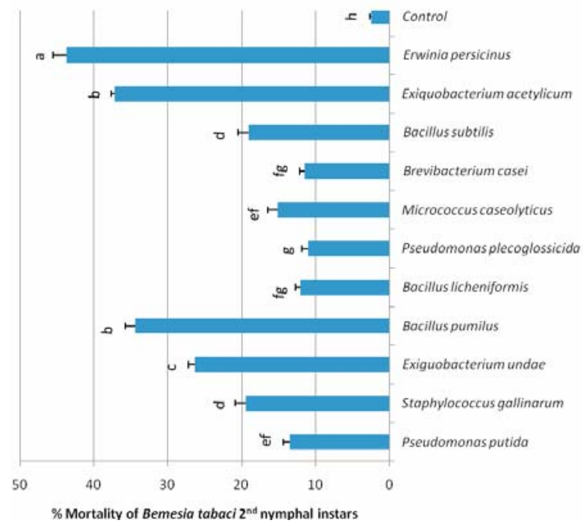

Figure 2. Percentage mortality of *Bemisia tabaci* 2nd nymphal instars (± SE), 3 days post application with bacteria. Percentages were arcsine transformed before analysis. Means with the same letter are not significantly different using LSD at 95 % confidence level.

bacterial isolates was recorded 5 days post treatment when compared with that recorded after 1 and 3 days, the treatments showed the same scenario within these three periods (Figs. 1- 3). Using *Erwinia persicinus* resulted in significantly (*F =58.8; df.=11,72; P<0.001*) higher mortality compared with other isolates (Fig. 3), and this was the only isolate that resulted in more than 50% mortality five days post application (Fig. 3).

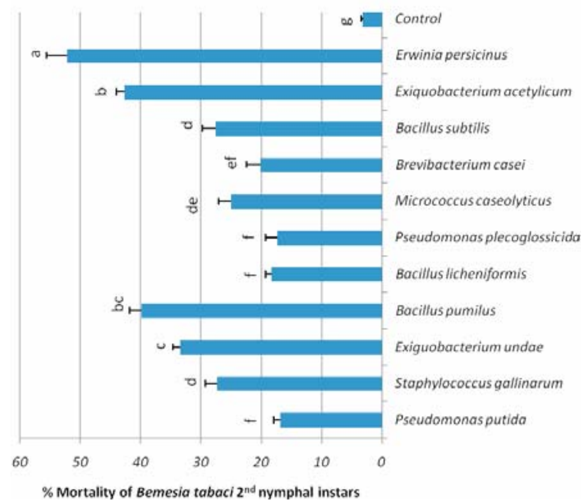

Figure 3. Percentage mortality of *Bemisia tabaci* 2nd nymphal instars (± SE), 5 days post application with bacteria. Percentages were arcsine transformed before analysis. Means with the same letter are not significantly different using LSD at 95 % confidence level.

Further studies using the three most promising isolates *Erwinia persicinus, Bacillus pumilus*, and *Exiquobacterium acetylicum* showed that mortality caused by these isolates did not increase remarkably with increasing densities of the bacterial cultures (Fig. 4). These three bacterial isolates resulted in mortalities ranging between 30 and 52% (Figs 1-3). For these isolates, mortality of 2nd nymphal instar *B. tabaci* increased with

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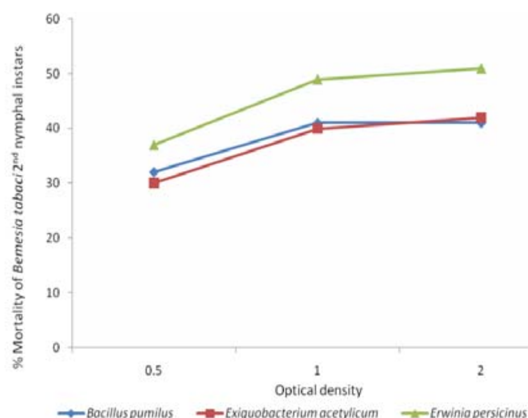

Figure 4. Mortality of *Bemisia tabaci* 2nd nymphal instars treated with three bacterial isolates at three different concentrations.

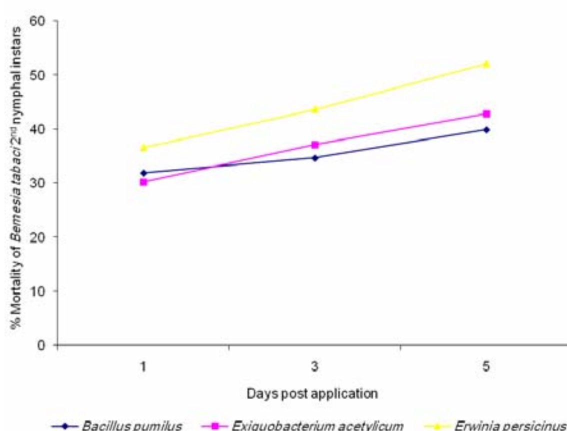

Figure 5. Time response mortality of *Bemisia tabaci* 2nd nymphal instars exposed to three bacterial isolates, isolated from whitefly adults.

time, and the most notable increase in mortality was observed with *Erwinia persicinus* from 36 to 52% (Fig. 5).

4. Discussion

Screening candidate biological control agent (BCAs) from a variety of microorganisms and environments is both difficult and laborious (Enya et al., 2007). Microorganisms that can grow on the phyllosphere may be better candidate BCAs than those that cannot (Andrews, 1992). Some of these bacteria are originally reside in plant tissues, mainly inside vascular tissues without doing harm to the plant, and they transfer to the whiteflies as they probe the vascular tissues of their host plants (Fukui et al., 1999; Kobayashi and Palumbo, 2000). This finding implies that cultivable whitefly-associated bacteria may be effective as BCAs against *B. tabaci*. In the present study, mortality towards *B. tabaci* 2nd nymphal instars was obtained, which exceeded 50% using *Erwinia percisinus* that was isolated from whiteflies collected from cauliflower plants (Ateyyat et al., 2009a). Another species of Erwinia, *Erwinia aphidocola* isolated from the homopteran pea aphid showed mild pathogenicity against aphids (Harada and Ishikawa, 1997).

Among the tested bacteria, *Exiquobacterium acetylicum* (43 % mortality) and *Bacillus pumilus* (40% mortality) exhibited mild pathogenicity towards *B. tabaci* 2nd nymphal instars. The mild pathogenecity of the tested bacteria may have resulted from the production of antimicrobial metabolites that affect the mutualistic bacteria such as the well-known *Buchnera* spp. For example, the antibiotic gallidermin is produced by the Gram-positive bacterium, *Staphylococcus gallinarum* (Kempf et al., 2000). Gallidermin exhibits a powerful bacteriocidic activity against Gram-positive bacteria (Hörner et al., 1990). Although competition between BCAs and other associated microorganisms is generally considered an important factor in reducing the suppression activity of BCAs (Weller, 1988), the interactions between BCAs and resident bacteria on *B. tabaci* are poorly understood because the structure of the bacterial community on whiteflies is also poorly understood.

In this study, the effect of the tested bacterial isolates against *B. tabaci* 2nd nymphal instars was evaluated using the highly susceptible stage of whitefly in a leaf dip bioassay. This technique is expected to be better than suspending bacteria isolates in sucrose and feeding them to adults through parafilm sachets used by Davidson et al. (2001). We observed mortality after 1, 3 and 5 days post treatment. After that, we stopped getting the mortality because it exceeded 20 % in the negative control treatment. The highest increase in mortality from the first to the fifth day was obtained by *Micrococcus caseolyticus* (from 5% to 25 %), followed by *Erwinia percisinus* (from 36% to 52 %). The trials to increase the concentration of the three bacteria species to enhance their effectiveness as biological control agent did not give a valuable increase.

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