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ORIGINAL ARTICLE

Ecological applications of *Pseudomonas* as a biopesticide to control two-spotted mite *Tetranychus urticae*: chitinase and HCN production

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Abstract

The two-spotted spider mite (*Tetranychus urticae* Koch) is an important pest of many horticultural crops. A study was conducted to evaluate the effect of three fluorescent *Pseudomonas* isolates obtained from rhizospheric soil of tomato (*Solanum lycopersicon*) in Agadir, Morocco: Q110B, Q036B and Q172B, as potential biological control agents for *T. urticae.* Both acaricide and repellent activities were assessed on homogenous adult mites. The acaricidal activity test evaluated five concentrations of bacterial suspensions: 0 (control), 10^2, 10^4, 10^6, 10^8, and 10^{10} cfu · ml^{-1}, while only the 10^{10} cfu · ml^{-1} concentration of each bacterium was used for the repellent bioassay. The mortality rate and repellentindex were recorded 24, 48 and 72 h after application. Results indicated that the survival rate of *T. urticae* was reduced ($p \leq 0.01$) by all three bacterial isolates compared to control. Within the 24–72 h time period the mortality rates ranged from 8 to 87%, 16 to 99%, and 13 to 89%, for Q110B, Q036B and Q172B isolates, respectively. The isolate Q036B (LC_{50} = 0.598 cfu · ml^{-1}) provided higher mortality rates than Q172B and Q110B with LC_{50} values of 90,846 and 169,585 cfu · ml^{-1}, respectively. Repellent activity was also the highest with *Pseudomonas* Q036B having a 71% repellence index at 48 h after application. Regarding the mechanism of action, all three isolates produced hydrogen cyanide, and exhibited protease and cellulose activities, although only Q036B and Q172B had potential chitinase action. Identification analysis showed that the isolates were either *Pseudomonas putida* (Q172B) or *P. fluorescens* (Q110B and Q036B). Our results indicate that the *P. fluorescens* isolate Q036B is a promising candidate for biological control of *T. urticae*, and has potential to contribute to an integrated pest management program to control this important pest. Then the fruits produced will be qualified as safe for consumers and the environment. The present work was customized to give support for policy decision makers as an agroecological potential meeting needs of industries and ecological balance.

Key words: biological control, biopesticide, chitinase, ecology, *Pseudomonas*, *Tetranychus urticae*

Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a polyphagous pest that is distributed worldwide. This pest can cause significant yield losses in many agricultural crops, including fruits, cotton, vegetables, and ornamentals (Neethu *et al*. 2015). Direct plant damage is due to feeding punctures. This pest pierces the epidermis and feeds on the contents of mesophyll cells that results in chlorosis, due to a decrease in total chlorophyll content and an eventual loss of photosynthetic capacity. Under heavy two-spotted mite infestations, the plant will often times die (Park and Lee 2002). Spider mites can infest more than 1,000 different plant species in more than 100 plant families (van Leeuwen *et al.* 2010). Adaptive strategies of spider mites are based on high fecundity of females and a female-biased offspring sex ratio, which can lead to a rapid population increase (Carey and Bradley 1982). In conventional chemical control management of *T. urticae*, broad spectrum acaricides are often sprayed to reduce population levels. However, they can also eliminate natural enemies, including predatory mites. Moreover, acaricides can cause the development of pesticide-resistant mite populations (Attia *et al*. 2012).

A definition of biological control is: "the use of living organisms to reduce damage caused by pests and diseases to tolerable levels". Biological control methods have become more and more necessary (Fiedler 2012) since some pesticides are not allowed and the use of others is restricted due to very low residue limits. Indeed, the use of biological control agents is the most cost effective method to control pests (van Lenteren 2012). However, the net benefits derived from biological control are difficult to quantify with any degree of accuracy except in a closed and totally controlled system (glasshouse).

Biological control agents such as parasites, predators, bacteria or fungi can be used as alternative strategies for management (Arzanlou *et al.* 2016; Hamza *et al.* 2016). Bacterial organisms, such as *Xenorhabdus* spp., *Yersinia entomophaga*, *Pseudomonas entomophila*, *Burkholderia* spp., *Chromobacterium* spp., *Streptomyces* spp., *Bacillus* spp. and *Saccharopolyspora* spp., have all recently gained commercial interest for production of numerous metabolites that act as potent insecticides (Ruiu 2015). Chitinase enzymes that are able to degrade chitin present in the cell walls of fungi and insect exoskeletons are thought to be one of the important metabolites produced by these bacteria. Thus, several pathogenic bacterial organisms have potential as biological control agents for various fungal and insect pests. Therefore, they can be used as an alternative to chemical pesticides (Rathore and Gupta 2015).

Fluorescent *Pseudomonas* species have also been shown to be effective in the biological control of the two-spotted spider mite pest. Aksoy *et al.* (2008) indicated that *P. putida*, isolated from tomato soils in Turkey, provided high mortality rates to two-spotted spider mites. Furthermore, *P. fluorescens* produces bacterial chitinases which are effective in controlling the mites by hydrolyzing their chitinous exoskeleton (Roobakkumar *et al.* 2011). Therefore, the objective of this study was to evaluate the efficacy of fluorescent *Pseudomonas* isolates, from tomato rhizospheric soil obtained in Agadir, Morocco, as a biocontrol agent against *T. urticae*.

Materials and Methods

All experiments were conducted in a growth room at 25±1°C, 16 : 8 h (L : D) photoperiod, and 55% relative humidity (RH), at the Laboratory of Integrated Crop Production Unit, National Institute of Agronomic Research (INRA), Agadir, Morocco.

Isolation and characterization

Pseudomonas bacteria were isolated from rhizospheric soil of tomato plants at the experimental farm of INRA, Agadir, Morocco. For isolation King's B medium (King *et al.* 1954) was used and fluorescent *Pseudomonas* were selected under UV light (360 nm). The selected bacteria were characterized according to their response to the following biochemical characteristics: Gram-positive/negative, motility, oxidase, catalase, arginine-dihydrolase and Leven production (Falkow 1958), glucose fermentation (Hugh and Leifson 1953), nitrate reduction, gelatin hydrolysis, gelatin liquefaction, and carbon source utilization (Bossis *et al*. 2000). Growth conditions such as temperature, pH and salt tolerance were also evaluated (Bossis *et al*. 2000). Fluorescent *Pseudomonas* isolates were identified using API 20NE test (BioMerieux, SA, France) (Sarma *et al*. 2012). Concentrations used in this study were obtained by adding an aliquot of each bacterial isolate to 100 ml of nutrient broth amended with 0.1% Tween 20 and incubated in an orbital shaker incubator at 28°C and 150 rpm for 24 h. After incubation, centrifugation was performed at 10,000 rpm. Bacteria cells were adjusted in 640 nm using a spectrophotometer (Optizen 3220UV/VIS double beam, Mecasys, South Korea) at five concentrations (10^2, 10^4, 10^6, 10^8 and 10^{10} cfu · ml^{-1}) (Zhang *et al*. 2014). Sterile distilled water was used for control.

Acaricidal activity

Three fluorescent *Pseudmonas* bacterial strains Q110B, Q036B and Q172B were assessed for their ability to cause mortality to homogeneous age adults of *T. urticae* on tomato (*Solanum lycopersicon*) leaves. The experiment was set up as a 3 (bacterial isolates) × × 6 (concentrations) × 3 (time intervals) factorial in a randomized complete block design with four replications and three runs. The population of *T. urticae* used was maintained for more than 30 generations on tomato crops. Fresh leaves of 'Prystila' tomato (Gautier seeds company, France) were collected from unsprayed plants growing in a greenhouse (INRA experimental farm, Agadir, Morocco). The leaves were first washed with tap water and rewashed with sterile distilled water, and then dried under a laminar flow hood.

The effect of bacterial isolates on *T. urticae* adults was studied under laboratory conditions using a leaf-dip bioassay (Bouharroud *et al.* 2007). A leaf cage was prepared from Petri dishes (9 cm) containing Whatman paper soaked in sterile distilled water. A 1.5 cm diam. hole was made in the lid of Petri dishes and covered with muslin. Tomato leaflets were dipped in each isolate and concentration combinations for 20 s. The treated leaflets were dried under a laminar hood then transferred to leaf cages. The control leaflets were dipped only in sterile distilled water. Fifteen *T. urticae* adults(male to female sex ratio: 1–1.1) were then transferred to treated leaflets. Four replicates for each leaf cage and concentration were used. The cages were incubated at 24±2°C with a photoperiod of 16 : 8 h (L : D), and *T. urticae* mortality rate was assessed 24, 48 and 72 h after treatment. The bioassay was replicated three times. Two-spotted mite mortality rates were corrected using Abbott's formula (Abbott 1925):

$$\text{CrrM } [\%] = \frac{\text{DMN} - \text{DMNC}}{\text{MTN} - \text{DMNC}} \times 100,$$

where: CrrM – corrected mortality, DMN – dead mite number, DMNC – number of dead mites in control, MTN – total mite number.

Repellent activity

This experiment measured the repellent activity of the fluorescent *Pseudomonas* isolates on *T. urticae* adults. The 10^{10} cfu · ml^{-1} concentration (which provided the highest acaricidal activity in the previous experiment) was used. The modified choice test described by Pascual-Villalobos and Robledo (1998) was used to determine *T. urticae* repellent activity. This procedure used two boxes (that contained either control or treated leaves) connected with a 10 cm long translucent hose (1 cm diam.) having a hole in the center that allowed the introduction of two-spotted spider mites (Fig. 1). Leaflets were immersed for 20 s in either a bacterial suspension (10^{10} cfu · ml^{-1}) or distilled sterile water, placed under alaminar flow hood until dry, and then placed in the corresponding box in the repellent apparatus. Twelve *T. urticae* adults (male to female sex ratio: 1 to 1.1) were then transferred gently through the hole pierced in the middle of the linked-hose and then sealed. This system allowed mites to move freely to the control or treated box.

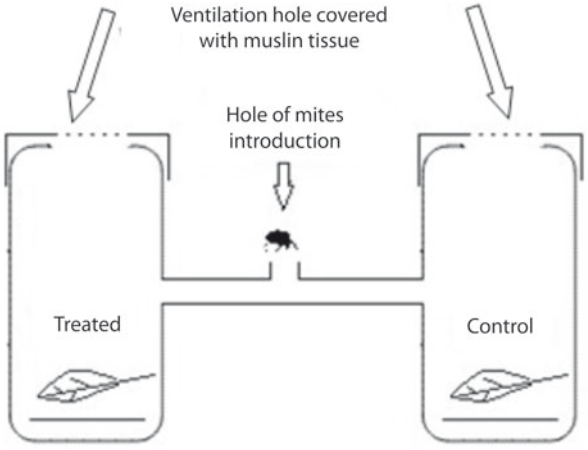

Fig. 1. Experimental apparatus adopted using tomato leaflets treated with either fluorescent *Pseudomonas* isolates or distilled water (control) for two-spotted mite repellent test

The experiment was set up as a 3 (bacterial isolates) × × 2 (concentrations) × 3 (time intervals) factorial in a randomized complete block design with four replications and three runs. Experiments were conducted in a growth room at 25±1°C, 16 : 8 h (L : D) photoperiod, and 55% RH. The migration of mites to one of the two linked boxes was determined at 24, 48 and 72 h after release. A repellency index was calculated using the formula of Pascual-Villalobos and Robledo (1998):

$$\text{RI} = \frac{\text{C} - \text{T}}{\text{C} + \text{T}} \times 100,$$

where: RI – repellency index, C – number of *T. urticae* adults in the control box, T – number of *T. urticae* adults in the treated box.

Mechanism of action of *Pseudomonas* strains

Chitinase production

The ability of an isolate to produce chitinase was determined as described by Cattelan *et al.* (1999) in medium composed of (g · l^{-1}): colloidal chitin prepared from crab shells (0.8); NH_4NO_3 (0.78); K_2HPO_4 (0.20); $MgSO_4 \cdot 7H_2O$ (0.20); $CaCl_2$ (0.06); NaCl (0.10); $Na_2MoO_4 \cdot 2H_2O$ (0.002); $ZnSO_4 \cdot 7H_2O$ (0.00024); $CuSO_4 \cdot 5H_2O$ (0.00004); $CoSO_4 \cdot 7H_2O$

(0.010); $MnSO_4 \cdot 4H_2O$ (0.003); $Na_2FeEDTA$ (0.028); H_3BO_3 (0.005); and, agar 15 (Sigma-Aldrich Chemical Co., USA). Magnesium sulfate and $CaCl_2$ were autoclaved separately and then added later to the medium after autoclaving. Biotin (5 $\mu g \cdot l^{-1}$) and ρ-aminobenzoic acid (10 $\mu g \cdot l^{-1}$) were filter-sterilized and added to the autoclaved medium. Each of the isolates was spotted on the chitin medium and incubated at 28°C for 72 h. A clear zone around the colony indicated chitin-solubilization by chitinase producing bacteria.

Cellulase production

M9 medium agar amended with 10 $g \cdot l^{-1}$ cellulose and 1.2 $g \cdot l^{-1}$ yeast extract was used to test for cellulase activity (Miller 1974). A clear halo after 8 days of incubation at 28°C was considered apositive response for cellulase production.

Proteolytic activity

Protease activities of *Pseudomonas* strains were determined according to the method reported by Jha *et al.* (2009). Skim milk agar was used and consisted of 5 $g \cdot l^{-1}$ pancreatic digest of casein, 2.5 $g \cdot l^{-1}$ yeast extract, 1.0 $g \cdot l^{-1}$ glucose, 100 $ml \cdot l^{-1}$ of 7% skim milk solution and 15 $g \cdot l^{-1}$ agar. Bacterial cells were inoculated by the spot method and incubated for 48 h at 28°C. After incubation, the plates were observed for the clear zone around the colony. A clear zone around the colony indicates protease production.

Hydrogen cyanide (HCN) production

To determine the production of HCN, bacterial isolates were streaked into King's B agar medium supplemented with glycine at 4.4 $g \cdot l^{-1}$. A piece of filter paper having 0.5% picric acid (yellow in color) and 2% sodium carbonate was positioned on the lid with Petri plates, then inverted, sealed with parafilm and incubated at 28°C for 96 h. Discoloration of the filter paper to orange/brown after incubation indicated microbial production of hydrogen cyanide (Bakker and Schippers 1987).

Statistical analysis

Data were subjected to ANOVA at $p \leq 0.01$ with interactions tested between the various factors for *T. urticae* mortality rates and repellent activities (Snedecor and Cocharn 1967). To determine the efficacy of *Pseudomonas* strains on *T. urticae*, mortality data were analyzed using Abbott's formula for acaricide activity (Abbott 1925), and Pascual-Villalobos and Robledo's (1998) equation for repellent activity. Resulting means were compared using Newman-Keuls test. The LC_{50} values and 95% confidence limit were calculated from probit regressions using the Polo-PC software (LeOra Software 1987). The index of significance for potency estimation "g" which is used for calculation of confidence limits at three probability levels – 90, 95, and 99%. If, at any of these levels, "g" exceeds 1.00, the values of the mean may lie outside the limits. As a safety feature, POLO-PC calculates confidence limits only when "g" is less than 0.5 at either the 90, 95, or 99% probability levels. The results of identification of each strain were interpreted using Apiweb® software (Biomerieux, France).

Results

No interactions ($p > 0.01$) were detected between bacterial isolates with different concentrations or time intervals, or concentrations with time intervals for *T. urticae* mortality rates and repellence. Therefore, data are presented only as main effects.

Fluorescent *Pseudomonas* isolation and characterization

The three *Pseudomonas* isolates evaluated (Q110B, Q036B and Q172B) had fluorescence production with diffusible yellowish-green pigment which fluoresced under ultraviolet light (360 nm). All strains were motile rods, testing positive for catalase and oxidase, and were Gram-negative. Q172B was positive for nitrate reduction, while Q110B and Q036B were negative (Table 1). Q110B and Q036B were positive for gelatin liquefaction, whereas Q172B was negative. All three strains were arginine deshydrogenase positive, and can use glucose as a source of carbon, but were negative for mannitol and sucrose utilization. The three strains can be grown in media ranging from pH 5 top H10 and at temperatures ranging from 4 to 44°C, except for Q172B which cannot grow at 4°C. All will grow under saline conditions (NaCl%), up to 6% for Q036B and Q110B and up to 5% for Q172B (Table 2).

Pure cultures of each isolate were identified using API 20 NE kit (Biomerieux, France). The results were interpreted using Apiweb® software and identified Q172B as *P. putida*, and Q036B and Q110B as *P. fluorescens.* These fluorescent *Pseudomonas* bacterial isolates were used in all subsequent work.

Acaricidal activity

All three *Pseudomonas* isolates provided significant amounts of mortality to *T. urticae* adults 24, 48 and 72 h after treatment, although specific isolates provided higher mortality rates than others (Table 3). The earliest deaths occurred within 24 h after application. The symptoms caused by *Pseudomonas* isolates on *T. urticae* were reduction of movement and the occurrence of brown-black coloration (Aksoy *et al.* 2008). The most rapid *T. urticae* deaths (24 h after treatment)

Table 1. Biochemical characteristics of fluorescent *Pseudomonas* bacterial isolates: Q172B, Q036B and Q110B

Pseudomonas isolate	Gram	Fl	Ox	Mt	Ca	N	Arg	L	Gl	Glu	Suc	Man
Q172B	–	+	+	+	+	+	+	–	–	ox	–	–
Q036B	–	+	+	+	+	–	+	+	+	ox/fr*	–	–
Q110B	–	+	+	+	+	–	+	–	+	ox	–	–

Fl – fluorescence, Ox – oxydase, Ca – catalase, Mt – motility, N – nitrate, Arg – arginine, L – leven, Gl – gelatin, Glu – glucose, Suc – sucrose, Man – mannitol, *ox – oxidation, fr – fermentation

Table 2. Effects of pH, temperature and NaCl concentration on growth of fluorescent *Pseudomonas* bacterial isolates: Q172B, Q036B and Q110B

Pseudomonas isolate	pH						Temperature [°C]						NaCl [%]						
	3	5	7	9	10	12	4	12	20	26	36	44	1	2	3	4	5	6	8
Q172B	–	+	+	+	+	–	–	+	+	+	+	–	+	+	+	+	+	–	–
Q036B	–	+	+	+	+	–	+	+	+	+	+	–	+	+	+	+	+	+	–
Q110B	–	+	+	+	+	–	+	+	+	+	+	–	+	+	+	+	+	+	–

"+"– growth and "–" – inhibition. Q172B is *P. putida*; Q036B and Q110B are *P. fluorescens*

Table 3. Effects of *Pseudomonas* isolates Q110B, Q036B and Q172B on *Tetranychus urticae* adult mortality rates

Pseudomonas isolate	Concentration [cfu · ml^{-1}]	Hours after application		
		24	48	72
Q110B	10^2	7.8 ± 5.9 a	31.5±22.7 a	53.7 ± 29.2 a
	10^4	13.1 ± 8.7 ab	38.7±10.9 a	67.8 ± 21.0 ab
	10^6	23.0 ± 12.9 abc	52.0±18.0 ab	79.0 ± 13.9 bcd
	10^8	23.1 ± 12.9 abc	55.9 ± 14.2 ab	84.4 ± 9.7 bcd
	10^{10}	34.6 ± 15.4 cd	64.8 ± 22.5 bc	87.7 ± 14.1 bcd
Q036B	10^2	16.0 ± 12.3 ab	42.4 ± 16.8 ab	69.2 ± 25.1 ab
	10^4	27.5 ± 17.8 bcd	80.1 ± 27.8 cd	94.4 ± 12.7 cd
	10^6	39.9 ± 13.3 c	85.6 ± 17.3 d	96.6 ± 7.8 cd
	10^8	41.2 ± 16.2 c	86.7 ± 14.0 d	97.6 ± 5.4 d
	10^{10}	61.9 ± 16.2 e	88.1 ± 18.7 d	98.8 ± 4.0 d
Q172B	10^2	13.5 ± 11.9 ab	30.8 ± 19.9 a	54.5 ± 19.0 a
	10^4	20.9 ± 13.2 abc	34.4 ± 18.2 a	75.1 ± 21.4 bc
	10^6	21.8 ± 8.6 abc	37.7 ± 23.2 a	86.8 ± 13.5 bcd
	10^8	29.9 ± 14.9 bcd	45.5 ± 21.7 ab	88.0 ± 14.4 bcd
	10^{10}	26.4 ± 10.4 bcd	47.9 ± 17.6 ab	89.0 ± 11.4 bcd

By column, the rates followed by the same letters are not statistically different at $p \leq 0.01$ according to the Newman-Keuls test

were obtained by Q036B which had a mortality rate of about 62% at 10^{10} cfu · ml^{-1} concentration, while Q172B and Q110B had approximately 26 and 35% mortality rates, respectively. At the highest concentrations, *T. urticae* mortality rates exceeded 50% 48 h after application, except for Q172B; and, more than 80% mortality was already achieved by Q036B at this time. The highest mortality rate (99%) was observed 72 h after application of Q036B. For the other two strains (Q172B and Q110B), the mortality rates were similar 72 h after application, and ranged from 54 to 89%. This was further confirmed by probit analysis indicating that the *Pseudomonas* isolate Q036B was the most toxic, and had an LD_{50} of 0.585 cfu · ml^{-1} compared to 90.846 and 169.585 cfu · ml^{-1} for Q172B and Q110B, respectively (Table 4).

Table 4. Log-dose probit mortality data for *Tetranychus urticae* adults tested with three *Pseudomonas* isolates

Pseudomonas isolate	LC_{50} [cfu · ml^{-1}]	Fiducial limits	Slope ± SE	g	Log (L)	N
Q036B	0.598	0.0 to 22.110	0.252 ± 0.037	0.162	–269.7	789
Q172B	90.846	0.195 to 2,530.947	0.192 ± 0.026	0.139	–447.5	838
Q110B	169.585	0.007 to 12,918.491	0.144 ± 0.026	0.242	–479.0	791

Log (L) – logarithm of the maximum value of the likelihood function; N – number of test subjects (individuals); g – index of significance for potency estimation

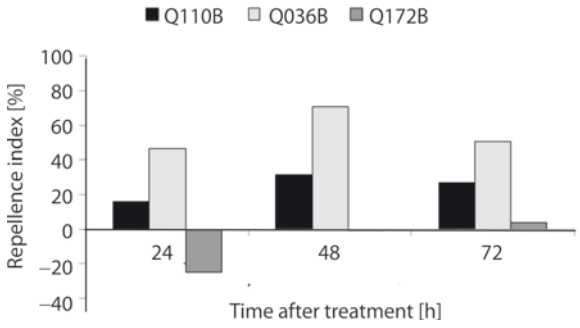

Fig. 2. Repulsive effect of *Pseudomonas* Q110B, Q036B and Q172B isolates on *Tetranychus urticae* adults. The repellence index (%) followed by the same letters do not differ at p ≤ 0.01 according to the Newman-Keuls test

Repellent activity

The three *Pseudomonas* isolates evaluated repelled *T. urticae* adults at 24, 48 and 72 h (Fig. 2). However, all three *Pseudomonas* isolates had a repellent effect only 72 h after application. The repellence index (RI) of the three isolates tested at 10^{10} cfu · ml^{-1} are shown in Figure 2 at 24, 48 and 72 h after application. At 72 h, both *Pseudomonas* Q110B and Q172B isolates had a low repellent effect on *T. urticae* adults with RI ranging from 4 to 27%, while *Pseudomonas* isolate Q036B provided the highest repellent effect of 50%.

Acaricidal mechanism

To elucidate the potential mechanism of action that could be involved in *T. urticae* adult mortality and repellency, the *Pseudomonas* isolates were characterized biochemically *in vitro* for chitinase, cellulase, and protease activity, and hydrogen cyanine production (Table 5). The chitinase plate tests of both *Pseudomonas* strains Q036B and Q172B had a clear halo surrounding the colony which confirmed their ability to induce chitin degradation; however, no chitinase activity was recorded for Q110B. For cellulase and protease activity, all *Pseudomonas* isolates tested positive, since a clear halo surrounded the colony in Petri plate observations for these enzymes. All fluorescent *Pseudomonas* isolates evaluated produced hydrogen cyanide, as indicated by discoloration of filter paper when incubated with picric acid (0.5%) and sodium carbonate (2%).

Discussion

The results of this study indicate that fluorescent *Pseudomonas*, especially *P. fluorescens* and *P. putida* have a high biological control efficacy against the phytophagous mite *T. urticae*. The genus *Pseudomonas* is known to have biocontrol potential for some pest species such as leaf folder insect, *Cnaphalocrocis medinalis* (Commare *et al.* 2002). Moreover, Aksoy *et al.* (2008) indicated that *P. putida* had a high efficacy and caused significant amounts of mortality to two-spotted spider mites. *Pseudomonas fluorescens* has also been shown to control the red spider mite (*Oligonychus coffeae*) (Roobakkumar *et al.* 2011). *Pseudomonas aeruginosa* has also been reported to control *T. urticae* (Poinar and Poinar 1998). Our study provides further evidence that *P. putida* and *P. fluorescens* will cause increased mortality of the adult of two-spotted mite, *T. urticae*, and have potential as biological control agents for this pest.

Table 5. *In vitro* chitinase, cellulase, protease activity, and hydrogen cyanide (HCN) production by fluorescent *Pseudomonas* isolates

Pseudomonas isolate	Chitinase activity*	Protease activity	Cellulase activity	HCN production
Q036B	+	+	+	+
Q172B	+	+	+	+
Q110B	–	+	+	+

*chitinase, protease and cellulase activity were detected by clear halo surrounding the colony; and, hydrogen cyanide detected by discoloration of filter paper when incubated with reagent

Fluorescent *Pseudomonas* bacteria act by several mechanisms for insect and mite pests, although the ability to degrade chitin is often considered the primary factor involved. Chitinolytic organisms, such as *Pseudomonas* sp. isolated from the rhizosphere, have been shown to have potential as biological control agents (Commare *et al.* 2002). Vodovar *et al.* (2006) reported that *P. entomophila* provides mortality to *Drosophila melanogaster* due to strong hemolytic activity, which involves proteins, such as lipases, chitinases and/or hydrolases. Furthermore, several researchers have indicated that bacterial chitinases were effective in providing control of insects and mites by hydrolyzing their chitinous exoskeleton (Broadway *et al.* 1998).Wilson *et al.* (2002) reported that bacterial hemolysins are exotoxins that attack blood cell membranes and cause cell rupture. This may also be involved in the pathogenicity of *Pseudomonas* strains on *T. urticae*, especially given the rapid mortality caused by Q036B. Additionally, Vodovar *et al.* (2006) indicated that pathogenic bacteria rely on a variety of cell surface-associated virulence factors that allow adhesion to the host surface to promote effective colonization. If applicable to *Pseudomonas* strains, this adhesion could enhance the incursion of proteases, chitinases, lipases and hydrolases through the cuticle, stigmas and body orifices of *T. urticae*, which can lead to the rapid death of the mite. Furthermore, the repellent effect of *Pseudomonas* strains can possibly be explained by the production of secondary metabolites, including volatile metabolites which kept mites from treated leaves in our study (Raaijmakers *et al.* 2002).

Biopesticides are key components of integrated pest management programs, and are often a way to reduce the amount of synthetic chemical products being used to control plant pests and diseases. Many horticultural crop growers throughout the world are now exploring their potential applicability due to ongoing pesticide restrictions. Results from our study further suggest that bacterial epiphytes and rhizospherics, some of which are of interest for use in the biological control of plant pathogens and pests, could also be examined for their potential use in insect pest management (Kupferschmied *et al.* 2013). This study indicated that although all three fluorescent *Pseudomonas* isolates provided significant death and repellence to *T. urticae*. *Pseudomonas fluorescens* isolate Q036B was the most promising candidate for biological control of *T. urticae*, and has potential to contribute to an integrated pest management program to control this important pest. Indeed, the fruits produced will be qualified as safe for both the consumer and the environment. This study will be a preliminary step for future work that will help policy decision makers support agroecological potential which will meet the needs of industries and maintain ecological balance.

Future research is planned to evaluate the impact of these *Pseudomonas* isolates under greenhouse conditions and to assess toxic and repellent effects to control other key pests of tomato (russet mite, whiteflies, leaf miners, thrips and aphids). On the other hand, the plant growth promoting rhizobacteria (PGPR) impact will be the most important study to ascertain the uses of these isolates as an alternative application to chemicals (fertilizers).

In addition, we propose molecular studies of these isolates. These may proceed from ascertaining key qualities of bacterial genomes and work towards sequence studies leading towards quantitative and qualitative measurement of the factors involved (Kearsey and Pooni 1998).

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