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Selection and screening of endorhizosphere bacteria from solarized soils as biocontrol agents against *Verticillium dahliae* of solanaceous hosts

Eleftherios C. Tjamos[1], Dimitrios I. Tsitsigiannis[2], Sotirios E. Tjamos[1], Polymnia P. Antoniou[1] and Panayiotis Katinakis[3]
[1]*Agricultural University of Athens, Laboratory of Plant Pathology, Votanikos 118 55, Athens, Greece (Phone: +30210 5294505; Fax: +30210 5294513; E-mail: ect@aua.gr);* [2]*Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI, 53 706, USA and* [3]*Laboratory of Molecular Biology, Votanikos 118 55, Athens, Greece*

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Abstract

Verticillium dahliae antagonistic endorhizosphere bacteria were selected from root tips of tomato plants grown in solarized soils. Fifty-three out of the 435 selected bacterial isolates were found to be antagonistic against *V. dahliae* and several other soilborne pathogens in dual cultures. Significant biocontrol activity against *V. dahliae* in glasshouse trials was demonstrated in three of 18 evaluated antagonistic isolates, provisionally identified as *Bacillus* sp. Although fluorescent pseudomonads were also isolated from root tips of tomato plants, none of the tested isolates exercised any significant antagonistic activity against *V. dahliae* in dual cultures. So these isolates were not tested in glasshouse trials in this study. Finally, two of the most effective bacterial isolates, designated as K-165 and 5-127, were shown to be rhizosphere colonizers, very efficient in inhibiting mycelial growth of *V. dahliae* in dual cultures and successfully controlling Verticillium wilt of solanaceous hosts. In glasshouse experiments, root dipping or soil drenching of eggplants with bacterial suspension of 10^7cfu ml^{-1} resulted in reduced disease severity expressed as percentage of diseased leaves (40–70%) compared to the untreated controls under high *V. dahliae* inoculum level (40 microsclerotia g^{-1} soil). In heavily Verticillium infested potato fields, experiments with potato seeds dusted with a bacterial talc formulation (10^8cfu g^{-1} formulation), showed a significant reduction in symptom development expressed as percentage of diseased potato plants and a 25% increase in yield over the untreated controls. As for their effectiveness in increasing plant height, both bacterial isolates K-165 and 5-127 produced indolebutyric, indolepyruvic and indole propionic acids. Both antagonists are considered as plant growth promoting rhizobacteria bacteria since significantly increased the height of treated plants compared with the untreated controls. Chitinolytic activity test showed that both isolates were able to produce chitinase. Testing rhizospheric and endophytic activity of the antagonists it was shown that although the bacteria are rhizosphere inhabitants they also preferentially colonize the endorhizosphere of tomatoes and eggplants. Fatty acid analysis showed that isolate K-165 could belong to *Paenibacillus alvei* while 5-127 to *Bacillus amiloliquefaciens.*

Introduction

Induced suppressiveness in solarized soils results in a prolonged positive effect of solarization in controlling soilborne pathogens such as *Fusarium oxysporum* f.sp. *vasinfectum*, *Rosellinia necatrix* and *Verticillium dahliae* (Katan et al., 1983; Greenberger et al., 1987; Tjamos and Paplomatas, 1988; Tjamos et al., 1991; Katan et al., 1989). Antagonistic rhizosphere bacteria

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survive solarization and contribute to the biological mode of action of this soil disinfestation procedure (Gamliel and Katan, 1991; Antoniou et al., 1995a). Having had a recent field observation that soil solarization induced a remarkable soil suppressiveness against *V. dahliae* in eggplant plantations, established in solarized soils several years after an application of solarization, research was focused on biological control factors from these specific solarized soils (Antoniou et al., 1995b). Berg et al. (1994) have demonstrated that naturally occurring rhizosphere bacteria are effective as biocontrol agents against *V. dahliae*. Zhengjun et al. (1996) reported rhizosphere and endophytic bacteria efficient in controlling Verticillium wilt of cotton. Furthermore, Berg and Lottmann (2000) reported that Verticillium wilt of oil seed rape could be controlled by a bacterial strain belonging to *Stenotrophomonas maltophilia*. Rhizosphere plant growth promoting rhizobacteria (PGPR) belonging to *Pseudomonas putida* and *Serratia marcescens* have been shown to be potential biocontrol agents against Fusarium wilt of cucumber (Liu et al., 1995). Huisman (1988) and Huisman and Gerik (1989) have shown that root tips are the actual sites of entrance of vascular pathogens in several hosts, thus indicating that the first place of interference of a successful bioantagonist must be mainly the area of the root elongation zone. The main objectives of this work were to: (a) isolate endorhizosphere antagonistic bacteria from tomato root tips and create a collection of *in vitro* antagonistic bacteria against *V. dahliae*, (b) selectively test their antagonistic activity against major soilborne pathogens *in vitro*, (c) evaluate their effectiveness as biocontrol preparations against *V. dahliae in planta* and (d) investigate potential antagonistic mechanisms of the most promising isolates against *V. dahliae* including auxin production and chitinolytic activity along with their efficiency in colonizing the rhizosphere and the vascular bundles of tomato seedlings and young eggplants.

Materials and methods

Isolation of antagonistic bacteria

Sandy loam soil samples were selected from solarized soils from NW Greece grown with tomatoes or eggplants and used as potting soil. Soil solarization experiments were carried out during summer by covering the soil with transparent polyethylene sheets for 4 weeks. Soil samples were obtained at least 3 years after a single application of soil solarization. Tomato seedlings grown under glasshouse conditions for 30 days were used as experimental plants. Tomato roots of uprooted tomato plants were washed with tap water and dipped in 100 ml of a 15% dilution of H_2O_2 for 2 min. They were rinsed three times in 100 ml of distilled–sterilized 0.1 M $MgSO_4$ and placed on Wattman filter paper for 2 min. One hundred root tip-samples of 0.5–1.0 cm length were removed with a sterilized needle, placed in a mortar with 1 ml of sterile distilled water (SDW) and macerated for 1 min with a pestle. The suspension was transferred to a McCartney bottle containing 9 ml of SDW and mixed in a vortex mixer for 5 min. Then, 10-fold dilutions were prepared and 200 µl of each dilution were plated in Petri dishes containing King's B medium (for *Pseudomonas* spp.) (King et al., 1954) and 523 medium (for *Bacillus* spp.) (Kado and Heskett, 1970). Petri dishes were incubated for 2 days at 30 °C. Bacterial colonies were transferred to NAG medium (Van Peer, 1990; Frommel et al., 1993).

In vitro *evaluation of antagonistic activity of endorhizosphere bacteria against soilborne pathogens*

A collection of 435 bacterial isolates was used for the evaluation of *in vitro* antagonistic activity against *V. dahliae* and several other serious soilborne pathogens on PDA (Garrett et al., 1966).

Beyond *V. dahliae*, 18 of the antagonistic isolates were checked for antifungal or antibacterial activity against several serious soilborne pathogens: *Pythium* sp., *Phytophthora* sp., *Rhizoctonia solani*, *Fusarium oxysporum* f.sp. *cucumerinum*, *Sclerotium rolfsii*, *Pyrenochaeta lycopersisci*, *Sclerotinia sclerotiorum*, *Thielaviopsis basicola*, *Phoma tracheiphila*, *Rahlstonia solanacearum*, *Erwinia carotovora* subsp. *carotovora, Clavibacter michiganensis* subsp. *michiganensis* and *Agrobacterium tumefaciens*.

Identification of bacterial antagonists

Preliminary identification of the bacterial antagonists was based on the colony morphology, shape of the cells, Gram reaction, oxidase and catalase reactions and their ability to form endospores (Lelliot and Stead, 1987). Further identification was also attempted by fatty

acid analysis carried out by The Belgian Coordinated Collections of Microorganisms.

Glasshouse experiments

Evaluation of biocontrol activity against Verticillium wilt of eggplants was carried out under glasshouse conditions. The research was focused on 18 isolates (17 antibiotic producing and one not-producing). The main criteria for the selection of potential antagonists were the ability to colonize the endorhizosphere and their antifungal activity against *V. dahliae in vitro*. Only bacteria provisionally identified as belonging to *Bacillus* spp. were used. *Pseudomonas* spp. isolates were not evaluated due mainly to limited antifungal activity against *V. dahliae*. Bacteria were grown in liquid cultures of nutrient broth and glycerol (NG) (Lelliot and Stead, 1987). Bacterial isolates were incubated in an orbital incubator at 180 rpm at $26 \pm 1\,^{\circ}$C for about 36 h. Bacterial suspensions were centrifuged at 8000 rpm for 10 min and resuspended by vortexing in 0.9% NaCl before treating the plants. One millilitre of sterile 50% glucose was added to each bacterial suspension (Leary and Chun, 1988). Microsclerotia of *V. dahliae* were produced in sucrose sodium nitrate (SSN) liquid medium (Tjamos and Fravel, 1995).

In planta glasshouse evaluation was performed with eggplants cv. 'Black Beauty' under controlled glasshouse conditions. A 1 : 1 mixture of sandy-loam soil and peat was used in the experiments. Bacterial isolates were applied at the one-leaf stage by dipping the root system of eggplant seedlings for 5 min in a 1×10^8 cfu ml^{-1} bacterial suspension. Ten eggplant-replicates were used per experiment while the experiment was repeated three times. Plants were grown throughout the experiment in separate pots. Individual plants were initially transplanted to 200 cm^3 pots ($7 \times 5 \times 6$ cm^3). Twenty days later (3–4 leaf stage) each plant was drenched with 10 ml of 1×10^8 cfu ml^{-1} bacterial suspension of the same antagonist. After 8 days each plant was transplanted to 650 cm^3 pots ($9 \times 9 \times 8$ cm^3) to allow the successful root colonization of bacterial antagonists. Bacterial density at this stage was 5×10^6 cfu ml^{-1}. Thirty days later (7–8 leaf stage), each plant ($10 \times 20 \times 20$ cm^3) was transplanted to plastic bag containing 3 l of soil infested with 40 *V. dahliae* microsclerotia g^{-1} soil. Control plants were treated with 0.9% NaCl. Disease severity, expressed as percentage of diseased leaves over the total number of leaves per plant, was periodically recorded for nearly 2 months after inoculation. At this stage, control inoculated plants had developed almost 100% diseased leaves. Plant height was recorded for each plant to check plant growth promoting activity of the evaluated bacterial isolates.

Population dynamics

Two bacterial isolates provisionally identified as *Bacillus* spp., designated as K-165 and 5-127 and found to be antagonists of *V. dahliae*, were evaluated as rhizosphere or endophytic colonizers of tomatoes and eggplants.

In tomato seedlings. Tomato cv. 'Early pack' seedlings grown under aseptic conditions in Petri dishes with wet filter paper were sprayed with a bacterial suspension of 1×10^8 cfu ml^{-1} at the cotyledon stage. The bacterial-treated seedlings were incubated for 6 h at 25 °C. The ability of the two bacterial strains to colonize the root tips of the seedlings internally was determined by surface disinfesting the seedlings in 15% H_2O_2 for 2 min, rinsing them three times in SDW, grinding the root tips in 0.05 M phosphate buffer at pH 7.02 using an autoclaved mortar and pestle, and plating them on B-523 medium.

In eggplants. In further experiments, spontaneous mutants resistant to 150 µg ml^{-1} rifampicin (Rif^R) were selected for K-165. Bacterial suspensions of Rif^R mutants of the strain K-165 were applied to eggplant cv. 'Black beauty' at the stage of fully expanded first leaf, at a concentration of 1×10^8 cfu ml^{-1} by root dipping for 5 min. At transplanting the treated plants were additionally drenched with 5 ml of bacterial suspension. Twenty days after the first application, 10 ml of bacterial suspension (1×10^8 cfu ml^{-1}) were drenched onto each plant. Rhizosphere and endophytic populations were determined at 15, 30, 45 and 60 days after the first application. To estimate rhizosphere populations, 0.5 g of rhizosphere soil (soil particles in close contact with roots about 1–5 mm thick) was carefully collected and shaken for 45 min. in 0.05 M phosphate buffer at pH 7.02 containing 0.02% Tween-20 and the suspension was then plated onto B-523 medium. Endophytic populations were determined after surface sterilizing the stem segments in 15% H_2O_2, for 2 min and rinsing three times with SDW. A 5-cm stem segment was cut at soil level and ground in 0.05 M phosphate buffer pH 7.02 with an autoclaved mortar and pestle and the

suspension was plated onto B-523 medium. Bacterial cell numbers were calculated on the basis of the approximate fresh weight of 5 cm eggplant stem segments at the time of sampling (cfu g^{-1} stem tissue).

Auxin production

In situ auxin production by biologically effective and ineffective bacterial strains was tested. By following the method of Brick et al. (1991) we used Luria broth tryptophan agar plates inoculated with toothpicks, overlaid with a nitro-cellulose membrane and treated with Salkowski's reagent.

Chitinolytic activity

Following the method of Gay et al. (1996) the chitinolytic activity of the effective *Bacillus* strains 5-127, K-165 along with the ineffective 5-102 strain was investigated. Bacterial isolates were plated on Luria medium supplemented with glycol chitin.

Field experiment

Bacterial isolates K-165 and 5-127 were evaluated under field conditions. Antagonists were applied to potato seed tubers in a dust formulation. Bacterial formulations were prepared in talc-gum xanthan (Kloepper and Schroth, 1981). Mass production of bacterial isolates for evaluating their biocontrol activity was obtained in a micro DCU-100 fermentor (B. Braun Biotech International) in potato-peptone culture medium and the population of the bacteria in the applied agent was equal to 10^8 cfu g^{-1} talc. The treatment was established at four experimental plots of 250 m^2 each severely naturally infested by *V. dahliae*. Each plot consisted of four K-165, four 5-127 and four untreated control rows each 80 m long. Potato seed pieces of cv Spunta were cut just prior to planting and treated with the biocontrol formulation at a ratio of 1 g of formulation per 92 g potato seed pieces (Wadi and Easton, 1985)

Results

In vitro *evaluation of the antagonistic activity of endorhizosphere bacterial isolates against* V. dahliae

A total number of 435 bacterial isolates were isolated from tomato root tips of tomato plants grown under greenhouse conditions. Twelve % (53 out of 435 isolates of the collection) produced antifungal compounds in dual cultures against *V. dahliae*. These 53 isolates, with the exception of 5-127 (Gram−), were Gram+, rod shaped, formed endospores, were positive to catalase and negative for a hypersensitive response to tobacco. Because the antibiotic activity was not a constant feature in all the initially selected isolates, *in planta* evaluation was carried out with 18 isolates (17 exhibiting antifungal activity and one not exhibiting). Data of Table 1 demonstrate that antagonistic activity against soilborne pathogens was a common feature in all isolates belonging either to Group K or to Group 5. However, *in vitro* antagonistic activity against oomycetes was absent. Isolate 5-102 had no effect on all tested pathogens except *R. solani* and *S. rolfsii.*

Evaluation of disease suppression under glasshouse

Data of Figure 1 (I, Best A) demonstrate that the endorhizosphere bacteria designated as K-165, K-160 and 5-127 were the most efficient in reducing disease severity expressed as percentage of diseased leaves in eggplants (40–70%) compared to the untreated controls nearly 2 months after transplanting to Verticillium-infested soil. The most effective isolates, grouped as Best B and Best C reduced the percentage of diseased leaves from 20% to 60%. These differences were statistically significant. On the contrary, differences among inefficient isolates, grouped as Worst A, Worst B and Worst C and control plants restricted to 0–30% were not statistically significant (Figure 1). The two antagonistic bacteria, that were selected as the most effective, reduced disease severity to a low percentage of diseased plants and significantly delayed appearance of first symptoms compared to the untreated control. On the other hand, *Bacillus* isolate 5-102 which did not prevent growth of *V. dahliae in vitro*, did not prevent disease development. Other bacterial isolates reduced disease incidence significantly, but did not show a constant behaviour during the three experiments.

Plant growth promoting activity

Data of Figure 2 clearly demonstrate that isolates 5-127 and K-165 were the most effective by promoting plant height 30–40% in treated plants as compared to the untreated controls (control – no fungus and *V. dahliae* – control but infected with the pathogen) 120 days after eggplant transplanting.

Table 1. In vitro antagonistic activity of 17 Gram+ and one Gram−(5-127), bacterial isolates in dual potato dextrose agar cultures against several soilborne phytopathogenic fungi and bacteria

Soilborne pathogens tested	Antagonistic activity* of 18 bacterial *Bacillus* isolates																	
	Group 5−									Group K−								
	5a	32b	39b	41b	102	108	127	148	149	109	124	127	141	158	159	160	165	167
Pythium sp.	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Phytophthora sp.	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Rhizoctonia solani	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fusarium oxysporum f.sp. *cucumerinum*	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+
Sclerotium rolfsii	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pyrenochaeta lycopersisci	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+
Sclerotinia sclerotiorum	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+
Thielaviopsis basicola	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−
Phoma tracheiphila	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+
Verticillium dahliae	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+
Clavibacter michiganensis subsp. *michiganensis*	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+
Rahlstonia solanacearum	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+
Erwinia carotovora subsp. *carotovora*	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+
Agrobacterium tumefasciens	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+

*The *in vitro* antifungal or antibacterial activity of 18 antagonistic endorhizosphere bacterial isolates was tested in dual cultures by using PDA as a nutrient medium. Group K and Group 5 correspond to the King's B and 523 nutrient medium, respectively, used for the initial isolation of the antagonistic bacteria from tomato root tips. +positive and −lacking antagonistic activity.

Bacterial identification

Fatty acid analysis carried out by The Belgian Coordinated Collections of Microorganisms (Wetenschapsstraat 8, B-1040 Brussels, Belgium) indicated that isolate K-165 possibly belongs to *Paenibacillus alvei,* while 5-127 to *Bacillus subtilis* – group possibly *Bacillus amyloliquefaciens.*

Auxin production and chitinolytic activity

Beyond K-165 and 5-127, most tested bacterial isolates of the collection produced a yellow to yellow–brown colour indicating production of the indolebutyric, indolepyruvic and indolepropionic acids. Similarly chitinolytic activity, observed under UV light as darkened clearing zones against the brightly fluorescing background, was evident in all tested isolates.

Population dynamics of K-165, 5-127 in solanaceous hosts

Both K-165 and 5-127 colonized the root tips of tomato and eggplant seedlings grown under aseptic conditions 6 h after treatment. Antagonists were traced in the root tips of the seedlings internally. No counting of bacterial cells was attempted.

Eggplants in glasshouses

By using Rif^R mutants of isolate K-165, it was shown at various stages of plant growth of eggplants grown under glasshouse conditions that this isolate is both a rhizosphere and an endophytic inhabitant. As shown in Table 2, K-165 was able to occupy the rhizosphere and the vascular tissue of eggplants at population levels of 10^4 cfu g^{-1} rhizosphere soil

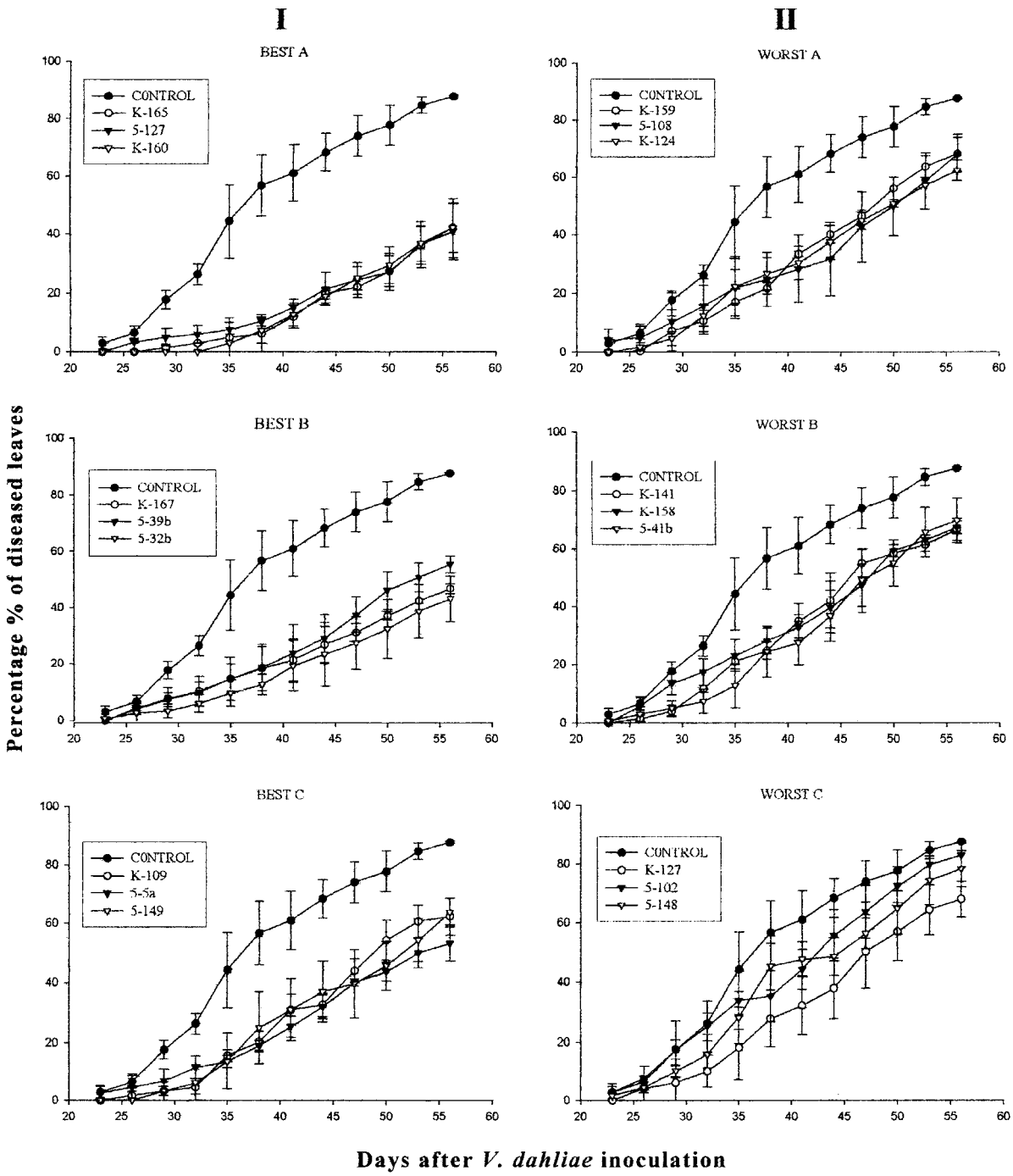

Figure 1. Effect of the most effective (I) grouped as BEST A, BEST B and BEST C and ineffective (II) grouped as WORST A, WORST B and WORST C endorhizosphere bacteria in reducing symptom development, expressed as percentage of diseased leaves compared to the untreated controls 57 days after eggplant transplanting. Experiments were carried out under controlled glass house conditions with 14 h photoperiod and 21 ± 3 °C air temperature. Means of 10 plants per treatment with three replications. Vertical bars indicate standard errors ($P > 0.05$ Fisher's LSD test).

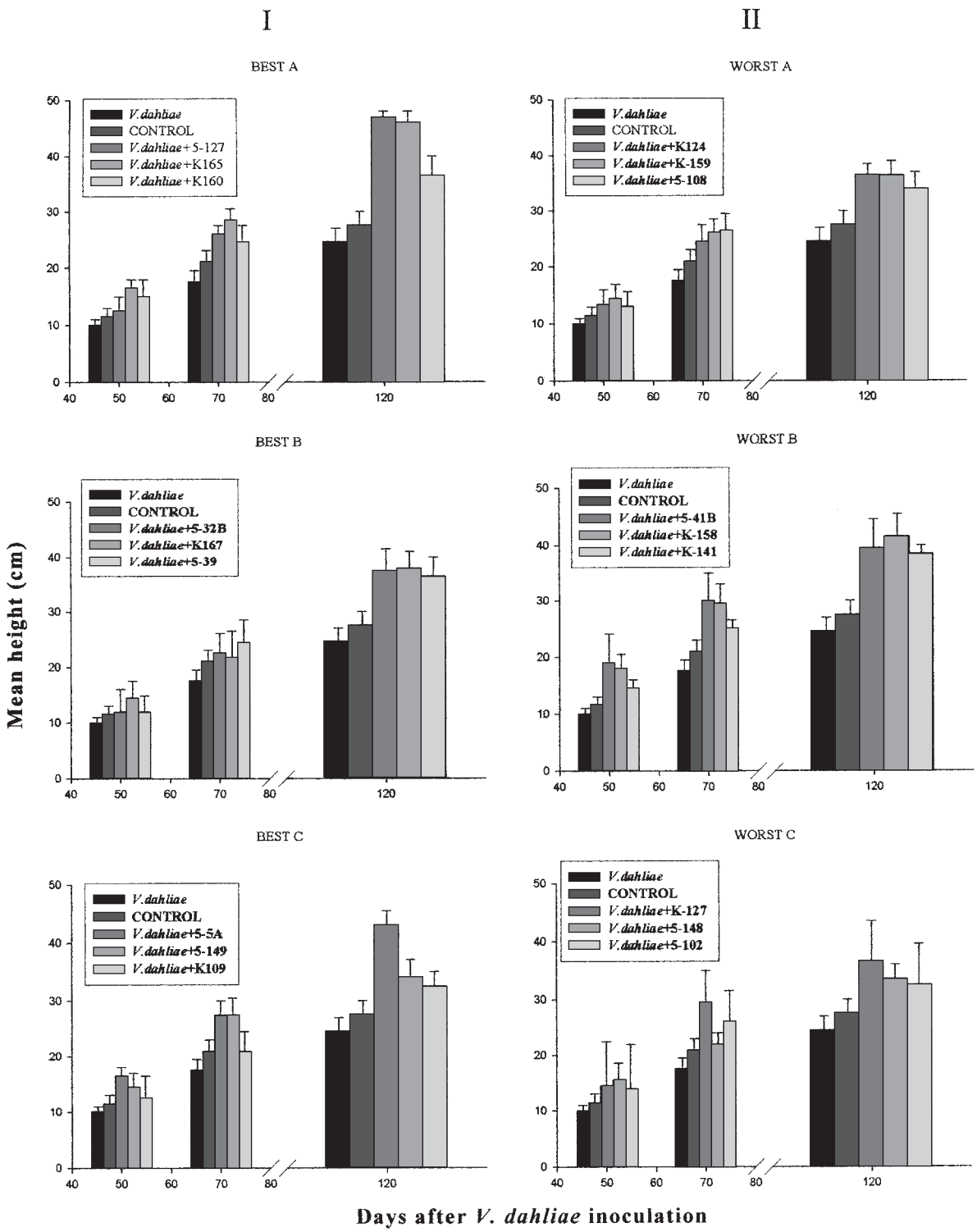

Figure 2. Effect of the most effective (I) grouped as BEST A, BEST B and BEST C and less effective (II) grouped as WORST A, WORST B and WORST C endorhizosphere bacteria in promoting plant growth response expressed as mean plant height in cm compared to the untreated controls (control – no fungus and *V. dahliae* – control but infected with the pathogen) 120 days after eggplant transplanting. Experiments were carried out under controlled glass house conditions with 14 h photoperiod and 21 ± 3 °C air temperature. Means of 10 plants per treatment with three replications. Vertical bars indicate standard errors ($P > 0.05$ Fisher's LSD test).

or 10^3 cfu g^{-1} stem tissue, with a diminishing trend with time.

Field evaluation

Isolates K-165 and 5-127 significantly delayed and eventually reduced symptom expression caused by Verticillium wilt in the potato field experiment 12–16 weeks (Table 3). Final symptoms at the harvesting stage showed that almost 40% of control plants were diseased. On the contrary, both antagonists reduced disease incidence by over 50% in treated compared to the untreated control plants.

Table 2. Fluctuation of rhizosphere and endophytic bacterial populations of isolate K-165 (Rif^R) 15–60 days after the first root dipping of young eggplants

Time after first bacterial application (days)	Rhizosphere* cfu g^{-1} soil	Endophytic** cfu g^{-1} stem tissue
15	$5.50 \times 10^4 \pm 1.3 \times 10^4$	$4.3 \times 10^3 \pm 1.6 \times 10^3$
30	$1.63 \times 10^4 \pm 6.1 \times 10^3$	$0.2 \times 10^3 \pm 0.2 \times 10^2$
45	$1.06 \times 10^4 \pm 4.0 \times 10^3$	$0.8 \times 10^2 \pm 0.2 \times 10^2$
60	$0.25 \times 10^4 \pm 0.4 \times 10^2$	$0.15 \times 10^2 \pm 0.2 \times 10$

*Rhizosphere populations are expressed as cfu g^{-1} rhizosphere soil and endophytic populations as cfu g^{-1} stem tissue. Data are means (±SE) of ten plants per treatment. **Endophytic populations were determined in cut 5 cm stem segments at soil level and ground in 0.05 M phosphate buffer pH 7.02 with an autoclaved mortar and pestle. The suspension was plated onto B-523 medium. Bacterial cells were calculated on the basis of the approximate fresh weight of 5 cm eggplant stem segments at the time of plant sampling (cfu g^{-1} stem tissue).

Discussion

One of the most effective and economical ways of controlling Verticillium wilt in mild climates in field-grown or covered crops is the application of soil solarization alone or in combination with reduced doses of methyl bromide (Antoniou et al., 1995b; Chet et al., 1990). Promising experimental data have shown that biological control of the disease could be an alternative approach, by applying selected antagonistic bacteria or fungi (Berg et al., 1994; Leben et al., 1987; Marois et al., 1982; Yuen et al., 1985; Zhengjun et al., 1996). The suppression of the disease by natural antagonists of the pathogen depends mainly on the ability of the antagonist to colonize the rhizosphere and on the production of various inhibitory substances, able to prevent disease caused by *V. dahliae* (Chet et al., 1990; Weller, 1988).

Our work demonstrated a frequent occurrence of endorhizosphere bacteria of *Bacillus* and *Pseudomonas* spp. in solanaceous hosts grown in solarized soils. This could be partially due to the increased soil temperatures facilitating survival of heat tolerant micro-organisms such as *Bacillus* or *Talaromyces* spp. (Tjamos and Paplomatas, 1988). Although 53 out of 435 bacterial isolates were *in vitro* antagonistic against various soilborne pathogens in general and *V. dahliae* in particular, significant biocontrol activity against *V. dahliae in planta* was demonstrated by only two out of 18 tested *Bacillus* isolates. In glasshouse experiments with eggplants, reduction in symptom development reached 40–70% of the leaves compared to the untreated controls under a high inoculum concentration of *V. dahliae* microsclerotia. This research demonstrates that Verticillium wilt could be significantly prevented

Table 3. Effectiveness of *Bacillus* sp. isolates K-165 and 5-127 in delaying or reducing symptom expression caused by Verticillium wilt and increasing potato tuber yield in the potato field experiment

Treatment	Percentage Verticillium infected potato plants[a]					Marketable potato yield in tonnes ha^{-1}[a]
	Time after seeding (in weeks)					
	12	13	14	15	16	
Control	3.79 ± 1.26	4.33 ± 0.87	7.70 ± 0.74	17.29 ± 2.11	39.92 ± 0.93	27.80 ± 0.79
K-165	1.50 ± 0.41*	1.69 ± 0.26*	2.05 ± 0.09*	4.31 ± 0.59*	15.67 ± 1.67*	33.72 ± 1.98*
5-127	1.56 ± 0.16*	1.38 ± 0.18*	2.00 ± 0.14*	6.39 ± 0.60*	15.82 ± 2.59*	34.65 ± 1.56*

Asterisks indicate statistically significant differences (Fisher's test) compared to the untreated control potato seeds.
[a]Values presented are means (±SE) of four replicates per treatment. Bacterial antagonists were applied as a talk formulation containing 10^8 cfu g^{-1} talc. Four experimental plots of 250 m^2 each per treatment were established at a potato field severely infested by *V. dahliae*. Each plot consisted of four K-165, four 5-127 and four untreated control rows each 80 m long. Potato seed pieces of cv. Spunta were cut just prior to planting and treated with the biocontrol formulation at a ratio of 1 g of formulation per 92 g potato seed pieces.

by selected endorhizosphere *Bacillus* spp. and is in agreement with other reports (Berg et al., 1994; Wadi and Easton, 1985; Zhengjun et al., 1996). Field experiments with potato plants demonstrated a significant reduction in symptom development and a 25% increase in yield, thus encouraging further studies with various other Verticillium susceptible crops.

This research proves that both strains are rhizosphere inhabitants and vascular tissue colonizers of solanaceous hosts. The magnitude of the bacterial populations reached as high as 10^4 cfu g^{-1} root soil or 10^3 cfu g^{-1} stem tissue. It is assumed that the ability of the bacterial antagonists to colonize both the rhizosphere and the vascular tissues could be one of the major factors of their effectiveness in controlling Verticillium wilt *in planta.* The selected bacterial isolates K-165 and 5-127 inhibited growth of *V. dahliae* and were successful biocontrol agents against Verticillium wilt.

As for the mode of action of these two bacterial isolates, beyond their antifungal or antibacterial activity against various soilborne pathogens, they produced auxins (Gay et al., 1996) and showed chitinolytic activity (Brick et al., 1991). However, all bacterial isolates tested had these properties indicating a lack of differentiation among effective and ineffective bacteria. Specific modes of action may include mechanisms contributing to the inhibition of *V. dahliae* microsclerotial germination, infection and its subsequent proliferation in the xylem vessels as it has been shown with fungal Verticillium antagonists (Tjamos and Fravel, 1995, 1997). Since these bioantagonists are present in the rhizosphere or inside the vascular tissues, they could also trigger induced systemic resistance against *V. dahliae* (Zehnder et al., 2001).

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