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Effects of *fluorescent Pseudomonads* for control of damping-off disease of cantaloupe caused by *Phytophthora drechsleri*

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Abstract

In this study, potential biocontrol agents including *Pseudomonas putida*, *P. aeroginosa* and *P. fluorescens* collected from the roots and rhizosphere of cantaloupe plants (*Cucumis mello L.*) sabze variety were tested against *Phytophthora drechsleri in vitro* and *in vivo*. After *in vitro* screening of 210 bacterial isolates of *Pseudomonas* spp, 16 isolates, which were the most effective antagonists, were selected for further studies. In laboratory experiments, most of the isolates inhibited mycelial growth of *P. drechsleri* by production of antibiotics and extra cellular compounds. Two isolates of *P. putida* (SCh) and *P. aeroginosa* (Zp) produced cyanide hydrogen. Seven of the 16 isolates, including *P. aeroginosa* (SG1 and Zh2), *P. putida* (SG2 and Zh1) and *P. fluorescens* (SSh, ZK2 and ZK1) produced siderophore and inhibited spore of *Geothricum candidum*. Isolates *P. fluorescens* (ZA, SSh and SU1), *P. putida* (SCh and SG2) and *P. aeroginosa* (SG1) produced Protease. Only one isolate of *P. putida* (SK1) produced cellulase. All tested bacteria produced indole acetic acid. In greenhouse conditions, isolates *P. fluorescens* (ZK2), *P. aeroginosa* (SG1) and *P. putida* (SG2 and ZH1) had the most significant effects on pathogen in seed and soil drenching treatments, respectively. The growth parameters (plant height, shoot and root dry-weight of seedlings) were significantly higher compared to the untreated control. Therefore, the antagonistic strains selected from the screening procedure provided significant protection against *P. drechsleri* through cantaloupe root colonization and could be applied by either seed or drench treatment as an alternative to chemical treatments of damping off disease of cantaloupe.

Keywords: Biological control, Cantaloupe, *Pseudomonas, Phytophthora drechsleri.*
Abbreviations: d_ day; h_ hour; NA_ Nutrient agar; KBA_ King's medium B Agar; PDA_ Potato dextrose agar; RH_ Relative humidity; w_ week; SKM_ Skimmed milk agar; CRD_ completely randomized design; DMRT_ Duncan multiple-ranges test.

Introduction

Cantaloupe (*Cucumis mello* L.) is one of the most important crops growing in Iran. Damping off disease caused by *Phytophthora drechsleri* can cause severe losses in cantaloupe plants in Iran (Shekari et al., 2006). *P. drechsleri* is a soil- borne pathogen and survive in the soils as oospores for several years as mycelia in plant debris (Jee et al., 2001). It has shown strong pathogenicity on cucurbits, tomato, pepper and ornamental plants that cause root rot and crown rot (Hyeong-Jin Jee et al., 2001; Lamour et al., 2003). This organism usually produces Zoospores that swim and infect susceptible tissues and readily dispersed across the field by rain or irrigation water (Shoun Zhang et al., 2010). Management of soil-borne pathogens in the field includes crop rotation, cultural practices, chemical control and use of resistant cultivars (Someya et al., 2000). The long-term survival of *P. drechsleri* even in the absence of susceptible host limits the effectiveness of crop rotation (Shouan Zhang et al., 2010). In addition, *P. drechsleri* has developed resistance to different fungicides and a number of fungicides have limited for use on cucurbits (Hausbek and Lamour, 2004). Therefore, alternative control measures, biological control using plant growth-promoting rhizobacteria (PGPR) have been regarded as attractive and need to be investigated (Shen et al., 2007). Several authors have reported the use of Pseudomonas species as a biological control agent against plant pathogens (Khalil and Al-Mughrabi, 2010; Manikandan et al., 2010; Masoud and Sharifi Tehrani, 2009). Research have indicated that *Pseudomonas* spp strains could reduce the incidence and severity of wilt of cotton (Oktay Erdogan and Kemal Benlioglu, 2010), Olive (Mercado-Blanco et al., 2004), Potato (Uppal et al., 2008) and improve growth parameters in these crops under greenhouse and field conditions. Also, *P. fluorescens* was shown to be effective in the biological control of Fusarium wilt of tomato (Manikandan et al., 2010) and citrus blue mould disease caused by *Penicillium italicum*. However, little is known about effectiveness of Pseudomonas species as biocontrol agent of damping off disease of cantaloupe. The objective of this study was to evaluate the efficacy of *fluorescent pseudomonads* for suppressing damping off disease of cantaloupe plants caused by *P. drechsleri* under laboratory and greenhouse conditions.

Results

Pathogenicity test

Four isolates of pathogen were isolated from infected cantaloupe plants and soil. One of them was found to be

Table 1. Effects of bacterial strains on fungal growth *in vitro* (percent growth inhibition)

Bacterial strain	Dual culture	Diffusible antibiotics	Volatile antibiotics
Control	0	0	0
P. aeroginosa Zp	24.5^{c}	35.6^{e}	15.3^{b}
P. aeroginosa ZH2	12.7^{gh}	37.9^{e}	12.0^{bc}
P. aeroginosa SG1	36.2^{a}	11.2^{h}	2.6^{e}
P. aeroginosa Sh1	18.6^{ed}	24.7^{g}	25.6^{a}
P. aeroginosa SE	13.3^{gfh}	37.0^{e}	7.6^{d}
P. fluorescens Sh2	19.1^{ed}	45.9^{cd}	1.2^{ef}
P. fluorescens SU1	18.1^{dfe}	37.0^{e}	1.0^{f}
P. fluorescens ZK2	31.4^{b}	37.0^{e}	6.5^{d}
P. fluorescens ZK1	24.9^{c}	37.0^{e}	10.9^{c}
P. fluorescens ZA	21.8^{ecd}	24.5^{g}	1.8^{ef}
P. fluorescens SSh	22.9^{cd}	85.6^{b}	15.3^{b}
P. putida SU2	18.1^{dfe}	47.3^{c}	2.8^{e}
P. putida SG2	38.8^{a}	91.8^{a}	15.3^{b}
P. putida SK1	11.2^{h}	35.3^{e}	1.3^{ef}
P. putida ZH1	38.3^{a}	31.5^{f}	11.2^{c}
P. putida SCh	17.0^{gfe}	43.5^{d}	5.3^{d}

Data are means of five replicates, Means in the column followed by different letter's indicate significant differences among treatments at 1% according to DMRT

Fig 1. Symptoms of damping off disease on cantaloupe plants (Left, control pathogen; right, control water)

significantly more pathogenic on cantaloupe and selected for laboratory and greenhouse experiments (Fig 1).

Antifungal activity in plate assay

Two hundred ten bacterial isolates were isolated from the rhizosphere and root of cantaloupe showing substantial inhibition zones against *P. drechsleri in vitro*. Of the 210 isolates, 16 (7.6% of total strains) were selected according to their high efficiency in *in vitro* antagonism, which was shown as inhibition zones in the dual-culture assay (Fig. 2). Based on biochemical, physiological and morphological properties tested, selected isolates were identified as different species of *Pseudomonas* including *Pseudomonas putida* (SG2, SK1, ZH1 and SCh), *P. aeroginosa* (Zp, Zh2, SG1, Sh1, SE and SU2) and *P. fluorescens* (SU1, ZK2, ZK1, ZA, SSh and Sh2). In dual-culture assays, *P. putida* SG2 with 38.8% and *P. putida* SK1 with 11.7% showed the highest and lowest growth inhibition percentages, respectively (Table 1 and Fig 2).

Antibiotic and volatile metabolites production

Results indicated that all bacterial isolates caused growth mycelia reduction of pathogen. The highest percent inhibition growth of pathogen was observed by *P. putida* SG2 (91.5%) followed by *P. fluorescens* SSh (85.6%). *P. aeroginosa* Sh1 with 25.6% growth reduction was the most efficient by producing volatile antibiotics (Table 1). Other isolates could not produce volatile antibiotics and do not have sufficient effect on pathogen compared with diffusible antibiotics.

Production of antifungal metabolites

Results of *in vitro* test by 16 bacterial isolates against the pathogen showed that strains exhibited different combinations of antimicrobial metabolites such as: protease, cyanide hydrogen, Siderophore, IAA and cellulase (Table 2). Howevers, only one strain, *P. putida* SK1 showed cellulase production. Two strains, *P. aeroginosa* Zp and *P. putida* SCh indicated positive reaction of cyanide hydrogen. Strains ZH2, SG1, ZK2, ZK1, SSh, and ZH1 inhibited *G. candidum* spores germination and produced siderophore (Table 2).

Efficiency of antagonistic bacteria in greenhouse condition

In soil treatment method, SG2, ZH1 and SSh isolates reduced the percentage of disease incidence to a range of 50-70% compared to control. *P. putida* SG2 was the most effective in controlling on control of damping off disease, but other isolates had lesser effects (Table 3). In the seed treatment method, bacterial isolates ZK2, SG1, ZH1 and SSh reduced disease 64%, 60%, 54% and 50%, respectively (Table 3). Isolates ZH1 and SSh reduced disease severity in both methods.

Table 2. *In vitro* activity of bacterial strains against *P. drechsleri*, by production of antifungal metabolites.

Bacterial strain	Protease	HCN	Siderophore	IAA[a]	Cellulase
P. aeroginosa Zp	−	+	−	+	−
P. aeroginosa ZH2	−	−	+	-	−
P. aeroginosa SG1	+	−	+	+	−
P. aeroginosa Sh1	−	−	−	+	−
P. aeroginosa SE	−	−	−	+	−
P. fluorescens Sh2	−	−	−	-	−
P. fluorescens SU1	+	−	−	+	−
P. fluorescens ZK2	−	−	+	+++	−
P. fluorescens ZK1	−	−	+	+	−
P. fluorescens ZA	+	−	−	+	−
P. fluorescens SSh	+	−	+	+	−
P. putida SU2	−	−	−	+	−
P. putida SG2	+	−	−	+++	−
P. putida SK1	−	−	−	+	+
P. putida ZH1	−	−	+	+	−
P. putida SCh	+	+	−	+	−

Data are mean of five replicates, [a] +: present, -: absent, +++: enhanced activity.

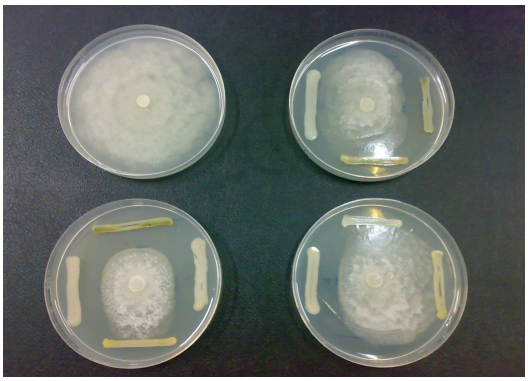

Fig 2. Effect of bacterial isolates on *P. drechsleri* on PDA by dual-culture assay (Up, left: Control and others consists pathogen and bacterial isolates

Effect of bacterial isolates on plant growth promotion

A total of 9 isolates induced statistically significant effects on plant growth compared with the non-treated control. Isolate Sh2 had the most effect on height and dry-weight of shoot by seed treatment method, but the isolate SG1 had more effect on dry-weight of root. In soil treatment method, isolate SSh had effect on all factors (Table 4).

Discussion

P. drechsleri is one of the yield limiting factors of cantaloupe across the world. Due to the soil-borne nature of the disease, use of chemical in controlling damping off disease is rarely successful. Inconsistencies in biocontrol under varying environmental conditions have been a common limitation of soil-borne pathogens. The present research was conducted to evaluate the efficacy of fluorescent pseudomonads against damping off of cantaloupe. Among PGPR, fluorescent pseudomonads occur commonly in the rhizosphere of plants and are the most diverse and ecologically significant group of bacteria for the control of soil-borne plant pathogens (Ellis et al., 2000; Oktay Erdogan and Kemal Benlioglu, 2010). This research has not investigated before in Iran. Of the 210 bacterial isolates, 16 isolates (7.6% of total strains) demonstrated *in vitro* antagonistic activity against *P. drechsleri*. These strains were selected from the rhizosphere of cantaloupe plants in different locations of cantaloupe-growing area. Most strains tested in this research exhibited no or weak antagonistic activity against the pathogen on PDA plates. This result is in agreement with other authors, so that *Psedomonas* spp. strains collected from cotton and weed rhizosphere inhibited the growth of *Verticilllium dahlia in vitro* (Oktay Eedogan and kemal Benlioglu, 2010; Zheng et al., 2011). In a dual-culture assay on PDA, the bacteria grew as well as the pathogen isolate. Of the 16 isolates, four were found to have highly inhibitory effects on mycelial growth of *P. drechsleri* (more than 30%), whereas others showed only mild activity (11.2 – 30%). *In vitro* assays showed that all bacterial isolates (except for isolate SG1) produced diffusible antibiotics and inhibited pathogen growth, whereas their ability to produce volatile antibiotics was weak (Table 1). Production of secondary metabolites such as antibiotics and enzymes by bacterial strains caused mycelia growth inhibition of *P. drechsleri* (Weller, 1998). Romanenko and Alimov (2000) reported that *P. fluorescens* isolate caused mycelia growth inhibition of *Bipolaris sorokiniana*. Also, these bacterial isolates produced secondary metabolites and inhibited the growth of *Pythium ultimum* (Jayaraj et al., 2007). Six strains produced protease, whereas one strain (*P. putida* SK1) produced cellulase and two strains (*P. putida* SCh and *P. aeroginosa* Zp) produced cyanide hydrogen

Table 3. Effects of seed and soil treatment with bacteria strains on Percentage disease incidence of cantaloupe seedlings under greenhouse experiment

Bacterial strain	Soil treatment	Seed treatment
P. aeroginosa ZH2	62.2^{Abc}	71.2^{abc}
P. aeroginosa SG1	81.2^{abc}	40.0^{cd}
P. fluorescens ZK2	76.2^{abc}	36.0^{d}
P. fluorescens ZK1	76.2^{abc}	77.5^{abc}
P. fluorescens SSh	47.5^{bcd}	50.0^{bcd}
P. fluorescens Sh2	81.3^{abc}	68.7^{acd}
P. putida SG2	30.0^{d}	76.2^{abc}
P. putida ZH1	50.0^{dc}	46.0^{bcd}
P. putida SCh	83.7^{ab}	85.0^{ab}
Control	100^{a}	100^{a}

Data are means of four replicates, Means in the column followed by different letter's indicate significant differences among treatments at 1% according to DMRT

Table 4. Effects of seed and soil treatments by bacterial strains on shoot length, dry weight of shoot and root of cantaloupe

Bacterial strain	Seed treatment			Soil treatment		
	DWR (g)	DWS (g)	MSL (cm)	DWR (g)	DWS (g)	MSL (cm)
P. aeroginosa ZH2	0.18^{cd}	0.46^{b}	17.3^{abc}	0.18^{b}	0.45^{ab}	23.2^{a}
P. aeroginosa SG1	0.35^{a}	0.36^{c}	18.2^{ab}	0.11^{e}	0.32^{c}	19.4^{b}
P. fluorescens ZK2	0.16^{d}	0.37^{c}	18.1^{ab}	0.12^{e}	0.33^{c}	17.0^{c}
P. fluorescens ZK1	0.24^{b}	0.47^{b}	16.8^{bc}	0.07^{f}	0.32^{c}	19.5^{b}
P. fluorescens SSh	0.17^{d}	0.38^{c}	17.4^{abc}	0.23^{a}	0.46^{a}	23.6^{a}
P. fluorescens Sh2	0.18^{cd}	0.49^{a}	18.9^{a}	0.14^{d}	0.43^{ab}	20.3^{b}
P. putida SG2	0.16^{d}	0.46^{b}	17.5^{abc}	0.17^{bc}	0.42^{b}	17.5^{c}
P. putida ZH	0.16^{d}	0.45^{b}	17.2^{abc}	0.16^{dc}	0.44^{ab}	20.6^{b}
P. putida SCh	0.19^{c}	0.32^{d}	15.8^{dc}	0.11^{e}	0.34^{c}	16.7^{c}
Control	0.05^{e}	0.25^{e}	15.1^{d}	0.05^{f}	0.25^{d}	15.1^{d}

Data are means of five replicates, Means in the column followed by different letter's indicate significant differences among treatments at 1% according to DMRT. DWR: dry weight of roots; DWS: dry weight of shoots; MSL: mean shoot length

(Table 2). Isolates *P. fluorescens* CHAO reduced black root rot of tobacco caused by the *Thieloviopsis basicola* (Visard *et al*., 1989). Strains ZH2, SG1, SG2, ZH1, SSh, ZK and ZK1 produced siderophore. Other researchers have reported induction of siderophore by *P. aeroginosa* on KB medium (Baudoin et al., 1988; Sunish Kumar et al., 2005). Our study indicated that different isolates applied as soil drench or seed treatment significantly reduced disease severity of damping off disease caused by *P. drechsleri* under greenhouse conditions. These results showed that five out of nine strains (SG2, ZH1, SSh, ZH2 and SG1) significantly reduced symptom expression (50 to 70%) under greenhouse conditions. Strain *P. putida* SG2 in soil treatment was the most effective in controlling the disease. Furthermore, this strain showed high efficiency *in vitro* as inhibition zones in dual-culture assay (Table 1), diffusible antibiotics, IAA and protease production (Table 1 and 2). Results indicated that the metabolites produced by these biocontrol agents have a direct role to play in reduction of diseases (Slininger et al., 2003; Khalil and Al-Mughrabi, 2010; Masoud and Abbas, 2009). Strain X16 of *Bacillus cereus* produced volatile substances that inhibited *in vitro* growth of *F. roseum* var. *sambucinum* (Sadfi et al., 2001). Shouan et al (2010) reported that treatment with PGPR induced significant levels of resistance against pathogens belonging to Oomycets including Phytophthora and sporulation of *P. capsici* was also significantly decreased by treatment with PGPR (Shouan et al., 2010). In the seed treatment method, isolates, ZK2, SSh, SG1 and ZH1 had significant effect on disease control. Isolate ZK2 was the most effective in reducing diseases under greenhouse conditions. Some researchers have indicated that the presence of the antagonist on plant seeds during the first stage of germination before the establishment of pathogen, significantly reduce disease severity, for instance seed treatment with *P. fluorescens* isolates reduced severity of Phytophthora blight caused by *P. capsici* (Sid et al., 2003). Growth parameters such as shoot length, dry shoot weight and dry-root weight were significantly higher in seed and soil treatment compared to untreated control. Isolates SSh and Sh2 had the most effects on three factors in soil and seed treatment, respectively (Table 4). Research has shown that many microorganisms from rhizosphere such as PGPR can have positive influence on plant growth and plant health (Zakira Naureen, 2009; Oktay Erdogan and Kemal Benlioglu, 2010). The role of *fluorescent pseudomonads* in promoting root and shoot growth of different crops has been demonstrated (Kamal et al., 2008). All isolates except ZH2 and Sh2 produced IAA, which suggest that bacterial strains could be used as plant growth promoters and improve plant health (Zakira Naureen et al., 2009; Naureen et al., 2005). Almost all of the bacterial isolates could promote shoot length, dry shoot weight and dry root weight compared to control plants. Use of fluorescent Pseudomonads against damping off cantaloupe not only suppressed the disease incidence and decreased the number of seedlings with damping-off symptoms, but also increased growth parameters of the plant. Further research is needed regarding the role of environmental factors in antibiotic synthesis modulation by *fluorescent pseudomonads* in order to improve the efficacy of bacteria strain in suppressing the *P. drechsleri* under field conditions.

Materials and methods

Culture media and organisms

P. drechsleri were isolated from infected cantaloupe seedlings of Markazi province, Iran. Pathogenicity of isolates was assessed on cantaloupe seedlings under greenhouse conditions (22- 26°C, 60-70% RH, 16 h light and 8 h darkness). Cantaloupe seeds were disinfected with 2% sodium hypochlorite for 15 min, rinsed with sterile distilled water and sown in pots (10-cm-diameter and 18-cm-height) containing steam-sterilized soil (including loam clay soil collected from the field with PH 7.9). All pots maintained in a glasshouse and plants were watered twice a week and once a week with the fertilizer solution (NPK 1:1:1). After 35 d, three mycelia discs (5-mm-diameter) of each isolate were placed at the edge of plant roots of cantaloupe seedlings. Control pots were without inoculum of pathogen. Pots were watered and after three w dead plants were recorded. Flourescent Pseudomonads were isolated from the rhizosphere of cantaloupe grown in fields at 10 locations of Markazi province, Iran from 2007 to 2009 (King et al., 1954). Bacterial isolates were stored in KBA medium at 4°C and subcultured at monthly intervals. After screening more than 210 bacteria isolated from rhizosphere and roots of symptomless cantaloupe in dual-culture test, 16 isolates with the best inhibition effects against *P. drechsleri* were selected for further experiments.

Antagonistic activity

The antagonistic effects of 210 bacterial isolates were tested for their ability to produce antifungal substance against *P. drechsleri* using a dual-culture assay on PDA medium. Four active bacterial isolates were spotted on the edge of the plates at equal distance (one cm). After 24 h incubation at 26°C, a single 5-mm-diameter mycelia disc of pathogen was placed in the center of the plates (with five replications for each one). As a control, a disc of *P. drechsleri* was placed at the center of PDA medium in a plate. Plates were incubated for one week at 26ºC in darkness and the radius of each fungal growth was measured. Relative growth inhibition was expressed as a percentage [(control-treatment)/control × 100] (Weller and Cook, 1986; Oktay and Kemal, 2010). Experiment was repeated twice. Bacteria with inhibitory potential were selected for further stages. According to inhibition zone produced, bacterial isolates were placed in five groups (without inhibition zone, 0-2.99 mm, 3-6.99 mm, 7-9.99 mm and ≥ 10 mm inhibition zone).

Production of volatile antibiotics

Two hundred µl of bacterial suspension (1×10^7 cfu/ml, count the number by Spectrophotometer) was placed at the center of a Petri-plate containing NA medium (merck, Germany), and a 5- mm disk of a 7-d-old pure culture of *P. drechsleri* was placed at the centre of another Petri-plate containing PDA media. Then, both half plates were placed face to face preventing any physical contact between the pathogen and the bacterial suspension. Plates were then sealed to isolate the atmosphere inside with parafilm to prevent loss of any volatiles formed (Zakira Naureen et al., 2009). In the control plates, bacterial suspension was replaced with sterile water. Plates were incubated at 27-29°C for 48 h and the percentage of inhibition zone was measured for each isolate as per to formula discussed previously. Experiments were repeated twice with four replicates for each treatment (Zakira Naureen et al., 2009; Fiddaman and Rossall, 1993).

Production of diffusible antibiotics

A 0.2 µm cellophane membrane was placed on PDA plates and Two hundred µl of antagonistic bacterial suspension (1×10^7 cfu/ml) were placed in the center of the plates. Plates were incubated at 27-29°C for 72 h, then the membrane was removed and a five mm disk of a pure culture of *P. drechsleri* was placed in the center of plates. Plates were incubated at 27-29°C for 120 h and the growth radius of the pathogen was measured. Bacterial suspension was replaced by sterile double-distilled water in the control plates. The experiment was repeated twice with four replicates for each treatment (Zakira Naureen et al., 2009; Kraus and Loper, 1990).

Protease production

Bacterial isolates were tested for production of protease by growing on SKM Agar (Chantawannakul et al., 2002). Plates were incubated at 27°C for 24 h. An ability to clear the SKM suspension in the agar was taken as evidence of the secretion of protease.

Cellulase production

Bacterial isolates were inoculated on cellulose medium (1 g K_2HPO_4, 0.5 g $NaNO_3$, 0.5 g KCL, 0.01 g $FeSO_4$ and 1000 ml distilled water)**.** A piece of paper (1×9 cm) was placed in a tube containing 9 ml of the cellulose solution and after inoculation with one loopful of each bacterial isolates, tubes were incubated at 25°C for three w.

Hydrogen cyanide production

Production of cyanide hydrogen was determined by growing bacterial isolates on NA medium at 27°C for 48 h in plates. Then, a filter paper was soaked in 5 mg of copper (II) ethyl acetoacetate, 5 mg of methylene bis (-n-n-dimethyl-aniline) per 2 ml chloroform (Castric and Castric, 1983) and placed on top of the plates. Plates were sealed with parafilm and after incubation at 28°C for 24 h, colour changes were examined.

Siderophore production

This experiment was done according to method described by Weller and Cook (1983). Firstly, KB media containing 0, 25, 50, 100, 1000, Mm of $FeCl_3$ were prepared. Then 100 µl of bacterial isolates were cultured on the surface of media and plates were incubated at 28˚C for 5 d. Finally a suspension of *Geotrichum candidum* in sterile water at concentration of 1×10^6 cfu/ml was sprayed onto the surface of media in Petri-dishes and plates were incubated at 28˚C for 72 h. Inhibition of mycelial growth of *G. candidum* around bacterial colonies indicated siderophore production.

Indole acetic acid production (IAA)

The production of IAA was determined as described by Bric et al. (1991). Bacterial isolates were inoculated in nutrient broth (peptone, 5g; yeast extract, 1.5g; beef extract, 1.5g; and NaCl, 5g; each per liter) without or with tryptophan (500 mg/l) and incubated at 30°C for 5 d. A 5-ml culture was removed from each tube and centrifuged at 10,000 rpm for 15 min. An aliquot of 2 ml supernatant was transferred to a fresh tube to which 100 µl of 10 mM orthophosphoric acid and 4 ml of reagent (1 ml of 0.5 M $FeCl_3$ in 50 ml of 35% $HClO_4$) were added. The mixture was incubated at room temperature

for 25 min, and the absorbance of developed pink color was read at 530 nm.

Greenhouse experiment

Nine isolates including *Pseudomons aeroginosa* (ZH2 and SG1), *P. fluorescents* (ZK1, ZK2, SSh and Sh2) and *P. putida* (SG2, ZH1 and SCh) were selected for greenhouse assays.

Seed-treatment

Cantaloupe seeds were soaked in a bacterial-methylcellulose suspension (10^8 cfu/seed) for 30 min and dried under a laminar flow hood. Control treatments consisted of non-treated dry-seed or seeds coated with 1% methylcellulose. Then seeds were sown in sterilized-soil infested with *P. drechsleri* at a concentration of 10^6 spores/g soil into 10-cm-diameter pots. A complete randomized design (CRD) with five replicates and three seeds in each pot were used in this experiment (Dileep Kumar and Dube, 1992).

Soil drenching

Both pathogen and antagonist were added to soil of pots at a concentration of 10^6 spores/g and 10^8 cfu/g soil (70 ml), respectively, and then seeds were sown in pots (Baudoin et al., 1988 and Weller and Cook, 1986). All pots were maintained in a greenhouse at 22-28°C, 60-70% RH, 16 h light and 8 h darkness. Plants (2-3 leaves) were watered twice a week and once a week with the fertilizer solution (NPK 1:1:1).

Diseases incidence assessments

Measurement of growth factors was performed 20 d after planting of seeds. The disease incidence was measured using the following formula:
Percent disease incidence = [(number of infected plants)/total number of plants] × 100
Finally, seedlings of all treatments were removed, washed with distilled water and placed in an incubator at 60°C for 24 h. In both methods, shoot length, dry weight of shoot and root were determined for each plant.

Statistical analysis

Experiments were designed as a CRD with five replications. All data analyses were conducted using the Statistical Analysis Software System (SAS Institute, Cary, USA, 1998), which included two plants per replicate for each treatment and the experiment was repeated twice. The means were compared by Duncan multiple-ranges test (DMRT) at $p \leq 0.01$.

Conclusion

The bacterial strains selected through the sequential screening procedure appeared to provide significant protection against the *P. drechsleri*, via cantaloupe root colonization. Bacterial strains could be applied by both soil and seed treatment for biocontrol of damping off disease of cantaloupe plants.

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