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RESEARCH ARTICLE

Bacterial inhibition of *Orobanche aegyptiaca* and *Orobanche cernua* radical elongation

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The ability of different bacteria to inhibit *Orobanche* radical elongation was examined. Seeds of *Orobanche aegyptiaca* Pers. and *O. cernua* Loefl. were induced to germinate with the Growth Regulator GR24 in the presence or absence of test bacterium. Radical lengths were estimated microscopically on a scale relative to seed length (0–5 seed lengths) after 5 days of incubation at 25°C. The results indicated that *Pseudomonas aeruginosa* QUBC1, *P. fluorescens* QUBC3, *Bacillus atrophaeus* QUBC16, and *B. subtilis* QUBC18 significantly inhibited radical elongation ($P \leq 0.01$) of both *O. aegyptiaca* and *O. cernua* relative to control radicals, whereas *Microbacterium hydrocarbonoxydans* QUBC11 and *Ochrobactrum anthropi* QUBC13 showed less inhibitory effects. Other bacterial isolates had no inhibitory effects. Bacterial isolates were identified using the universal method in addition to morphological and biochemical features. The establishment of the inhibitory effect of the most promising isolates, *B. atrophaeus* QUBC16 and *P. aeruginosa* QUBC1 on radical elongation of both *Orobanche* spp. is a step towards utilizing such bacteria as biocontrol agents against *O. aegyptiaca*, *O. cernua*, and potentially other *Orobanche* species.

Keywords: radical elongation; *Orobanche*; *Pseudomonas*; *Bacillus*; biocontrol; GR24

Introduction

Broomrape (family *Orobanchaceae*, *Orobanche*; Feinbrun-Dothan 1978) is an obligate plant parasite. The genus *Orobanche* comprises more than 150 species most of which grow around the Mediterranean region (Kreutz 1995). It is also widely distributed in climates similar to that of the Mediterranean region (Linke, Sauerborn, and Saxena 1989). In nature, germination of *Orobanche* seed takes place only when the seed comes in contact or close proximity to the root of a host plant (Sauerborn 1991; Kreutz 1995). Crop damage and losses caused by *Orobanche* can reach 100%. Different measures including cultural, biological, mechanical, and chemical are being applied to control these weeds (Kreutz 1995). Several factors contribute to the inefficiency of these methods in controlling *Orobanche* weeds; the parasite produces thousands of viable seeds that can remain dormant in the soil for several years and seed dormancy is only broken by the presence of host plant root in the vicinity of the seed. Other contributing factors are the microscopic size of seeds

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which allows them to spread to new agricultural fields, and the ability of *Orobanche* to grow on several crop and noncrop plants.

In recent years, increasing interest has been devoted to biological control of Broomrape. Biocontrol seems to be a promising approach since it allows use of diverse biological agents; for example, the insect *Phytomyza orobanchia* Kalt. is responsible for reducing seed production in *O. crenata* Forsk. to 50%. The fungi *Fusarium oxysporum* and *F. arthrosporiodes* are known to attack Broomrapes (Sauerborn 1991; Amesellem et al. 2001; Cohen, Amsellem, Lev-Yadun, and Gressel 2002). In addition, the biocontrol arsenal may be expanded to include viruses, bacteria, nematodes, and others that have the potential to act as *Orobanche* biocontrol agents.

Previous *in vitro* studies (Barghouthi, Faqieh, and Badrieh 2000), showed that some of the tested bacteria (*Enterobacter* sp. QUBC20 and *Serratia marcescens* QUBC6) may enhance *in vitro* germination of *Orobanche* seeds in the presence of the Growth Regulator GR24. GR24 is a synthetic strigolactone analogue (Mangnus, Dommerholt, de Jong, and Zwanenburg 1992). Strigolactones and flavonoids are secondary plant exudates that trigger secondary signals in parasitic plants such as *Striga* and *Orobanche* (Steinkellner et al. 2007). Strigolactones play a role in regulating above-ground plant architecture and in communication with other underground organisms (Umehara et al. 2008; McSteen 2009). They act as secondary signals which rhizotropically direct the parasitic germ tube to finding host root (Sauerborn, Stövera, and Hershenhornb 2007). Other bacterial isolates (*Stenotrophomonas maltophilia* QUBC61, and unidentified isolates QUBC67, and QUBC68) showed no significant inhibition of radical elongation. Zermane, Souissi, Kroschel, and Sikora (2007) have shown that *P. fluorescens* Bf7-9 can suppress the pre-emergence of *O. foetida* Poir. and *O. crenata* when tested in pot experiments.

In this study, the inhibition of radical elongation (germ tube) by different bacterial isolates is described for the two *Orobanche* species; *O. aegyptiaca* Pers. and *O. cernua* Loefl.

Materials and methods

Isolation, culture, and storage of bacteria

Soil samples (25 g each) were collected from an *Orobanche aegyptiaca* Pers. infested lentil field (Terra rossas brown rendzinas soil, clay with particle size <0.002 mm, pH $\sim$7.4, 80% water retention, high iron and organic matter, Kauber, Ramallah, West Bank, Palestine; Table 1). An area showing the least number of *O. aegyptiaca* shoots while surrounded by highly infested host plants was selected for sampling. The assumption was that bacteria capable of controlling *O. aegyptiaca* will reduce or eliminate shoots of *O. aegyptiaca* in that vicinity. Three bacterial isolates (L1, L2, and L3) were obtained. Similarly, another sample was selected from a *Vicia faba* L. field (Terra Rossas pale brown, pH $\sim$7.4, <0.002 mm high iron and water retention, Kauber; Ramallah, West Bank, Palestine; Table 1), where bacterial isolates F1 and F2 were obtained (Table 1).

Samples of healthy or diseased *O. cernua* plants (diseased shoots and tubercles on apparently healthy tobacco host plants) were collected from an infested tobacco field (soil known locally as Grumusols; coarse sandy >0.2 mm, pH $\sim$7, water retention

Table 1. Bacterial isolates utilised in this study.

Bacterium	Source of isolate	Year collected
Acinetobacter baumannii HK 89	*O. aegyptiaca* diseased juvenile shoot, Tomato field	1998
Agrobactrum tumefaciens HK13	A tuber on tobacco root	1998
Bacillus atrophaeus QUBC16	Autoclave test spores	1998
B. subtilis QUBC 18	Clinical stool sample	2007
Enterobacter sp. QUBC20	*O. aegyptiaca* shoot, tomato field	1998
E. cloacae QUBC67	*O. cernua* internal tissue of diseased shoot	1998
F1 and F2	Terra Rossas pale brown, pH 7.4, <0.002 mm high iron and water retention, sample from *Vicia faba* L. field, Kauber village	2001
L1, L2, and L7	Terra-Rossas brown Soil sample, Lentil field Kauber village	2001
Microbacterium hydrocarbonoxydans QUBC11	*O. cernua* diseased underground shoot	1998
Ochrobactrum anthropi QUBC13	*O. cernua* diseased underground shoots on apparently healthy tobacco plant	1998
Pantoea agglomerance HK1	*O. aegyptiaca*, tomato field	1998
Pseudomonas aeruginosa QUBC1	Melon root	2001
P. aeruginosa QUBC2	AL-Maqased, Local Hospital	2007
P. fluorescens QUBC3	*Phalaris* sp. Weed root	2007
Serratia marcescens QUBC6	Insect path inside mature *O. aegyptiaca* shoot, tomato field	1998
SH2,SH3,SH4,and SH5	Melon root	2001
Staphylococcus epidermidis QUBC21	A gift, M. Ayesh, Medical Laboratory Sciences, Al-Quds University	1998
Stenotrophomonas maltophilia QUBC61	*O. aegyptiaca* shoot	1998

~25%, Ya'bad, West Bank, Palestine). Grumussols is known as Exerert according to the Soil Survey Staff (1999). Healthy or diseased tissue was surface sterilized and ground in a blender (0.5 g tissue/ml of sterile tap water). The blender was surface sterilized before and after each sample.

Small weed samples were surface sterilized and aseptically dissected to expose internal tissues. A bacteriological loop was used to transfer and streak bacteria from the internal tissue to a nutrient agar plate. *Orobanche* shoots and seed pods showing insect tracks were aseptically cut to expose a long section of the track. A bacteriological loop was used to transfer and streak track material to a nutrient agar plate (Table 1).

Based on claims that Broomrapes grow poorly in fields that have been cultivated with honeydew melon (*Cucumis* sp.), five 3-week-old seedlings (grown in Terra Rosa Garden, Ramallah, West Bank, Palestine) were used to investigate the bacteria associated with roots of honeydew melon. Roots (5 g) were washed under tap water, shredded aseptically in a kitchen blender for 2 min in 50 mL sterile normal saline

(0.85% NaCl) and allowed to settle for 5 min. Three millilitres of each prepared soil or plant tissue suspension were centrifuged for 2 min at 3000 rpm to remove debris (Hettich Zentrifugen model MIKRO 12–24 microcentrifuge) and 1.5 mL of the supernatant was then centrifuged for 3 min at 14,000 rpm. Each pellet was resuspended in 1 mL 0.85% NaCl which was used to prepare 10-fold serial dilutions and 100-μL aliquots from each dilution were spread on Nutrient (supplemented with 0.5 μg mL^{-1} cyclohexamide) or Mac Conkey agar plates. Five isolates were obtained (QUBC1, SH2, SH3, SH4, and SH5; Table 1).

The cultured plates were incubated overnight at 25°C. Different types of colonies were selected based on colony shape, size, and colour. Each isolate was checked for purity on Nutrient (without cyclohexamide) or Mac Conkey agar plate after an overnight incubation at 25°C. A total of more than 100 bacterial isolates were obtained from soil samples, melon roots, healthy *Orobanche* shoots, diseased shoots showing abnormal coloration, brown spots, and tracks of insect larvae.

Bacteria obtained from other sources were included to avoid research bias and the possible exclusion of potentially important bacterial biocontrol agents. Preliminary results indicated the efficacy of other bacteria as biocontrol agents: *Pseudomonas aeruginosa* QUBC1 and *Bacillus atrophaeus* QUBC16.

P. aeruginosa QUBC2 (a clinical isolate) was solely used to confirm the results obtained with *P. aeruginosa* QUBC1 and was not utilised any further. Another clinical isolate, *B. subtilis* QUBC18 was introduced to support the results obtained with *B. atrophaeus* QUBC16. *Staphylococcus epidermidis* QUBC21 was included to represent the coccoid Gram positive bacteria. Table 1 lists the 24 bacterial isolates utilised in this study. For long-term storage, sterile glycerol was aseptically added to overnight bacterial cultures to a final glycerol concentration of 25%. After mixing, 1-mL aliquots were dispensed into screw cap sterile 2-mL cryogenic tubes and stored at −70°C.

Bacterial cultures and culture media

Pure bacterial cultures were inoculated into 2-mL Luria-broth prepared in 12-mL sterile Falcon® test tubes that were subsequently incubated overnight at 25°C. Overnight cultures were used for storage, identification, or experimentation as detailed below.

Seed preparation for bioassay of bacterial activity

Orobanche aegyptiaca seeds (lentil field, Kauber, Ramallah, West Bank, Palestine) or *O. cernua* seeds, obtained from an infested tobacco field in Ya'bad, Jinin, West Bank, Palestine, were collected from mature Broomrape plants. About 30 surface sterilized seeds were aseptically sprinkled on top of each Glass Fibre Filter Paper (Whatman GF/C) square (1 × 1 cm) that was placed on top of a lining GFFP pad inside 9-cm Petri dishes. Three mL of sterile bottled Jericho water (JW) were used for wetting. Sealed plates were incubated upside down in the dark for 7 days at 25°C. Before autoclaving, JW contained per litre: HCO_3, 298 mg; Ca, 69.7 mg; Cl, 49.4 mg; Na, 23.9 mg; SO_4, 21.5 mg; Mg, 20.2 mg; NO_3 19.14 mg; K, 2.8 mg; F, 0.2 mg; PO_4, and 0.08 mg; pH 7.8.

Induction of seed germination and radical measurement

Preconditioned Broomrape seeds were used on the seventh day in experiments designed for *in vitro* testing of seed germination and radical elongation. The GR24 (obtained from Binne Zwanenburg, Department of Organic Chemistry, Radboud University Nijmegen, The Netherlands; Mangnus et al. 1992) stock solution was prepared by dissolving 1 mg in 1 mL acetone and stored at -20°C in the dark. Freshly prepared working GR24 solution that was used throughout this study contained 2 μg mL^{-1} of GR24 in autoclaved JW. GFFP squares carrying the preconditioned seeds were aseptically transferred to test plates containing sterile GFFP pads that had been wetted with 3 mL of the working GR24 solution (positive control), 3 mL of JW containing 6 μL acetone (negative control), or 3 mL of JW containing GR24 and appropriate bacterial concentration. The plates were sealed with parafilm and incubated upside down in the dark for 5 days at 25°C. Seed germination and radical elongation was examined with a dissection microscope (Stereozoom 7, Bausch & Lomb Optical Co.). Since radicals were twisted (*O. aegyptiaca*) or curly (*O. cernua*), their length was estimated on a scale relative to seed length (usually from 0 to 5 seed lengths). Digital micrographs of seeds were obtained with a bright field compound microscope (Kruess Optronics, Hamburg, Germany; 40 ×) using a digital camera (Casio, Exilim, 12.1 mega pixels, Tokyo, Japan). Photos were then examined on a flat 17″ monitor. Radicals were estimated with the aid of a ruler. Measurements were verified by printing enlarged photographs and using a thread to trace the seed and the radical, the thread was then measured using a ruler. The length of radical was divided by the length of the seed to obtain radical lengths expressed in seed lengths. Since the average seed length of *O. aegyptiaca* is 0.25 mm (Hershenhorn et al. 1998) and 0.3 mm for *O. cernua* (Federal Noxious Weed Disseminules of the U.S. 2009), the following formula was applied to transform the relative length of radicals to absolute lengths in millimetres:

$$\textit{O. aegyptiaca}\ \text{Radical Length (mm)} = \text{Estimated radical length expressed in seed lengths} \times 0.25\ \text{mm};$$

$$\textit{O. cernua}\ \text{Radical Length (mm)} = \text{Estimated radical length expressed in seed lengths} \times 0.3\ \text{mm}$$

Effect of bacteria on radical elongation

To test the effect of bacteria on radical elongation, overnight bacterial broth cultures (18 h at 35°C) prepared in 15-mL screw-cap tubes containing 5 mL Luria broth were shaken on a nodator. One half millilitre of the culture was diluted 10-fold with Luria broth and incubated for 2 h at 35°C with no shaking. Bacteria were collected in sterile microfuge tubes by centrifugation for 1 min at 12,000 rpm. The supernatant was discarded and the bacterial pellet was resuspended in 1 mL of 2 μg GR24 mL^{-1} of sterile JW. Bacterial suspensions were then diluted in GR24 to give desired absorbencies, $A_{600nm} = 0.06$, 0.12, or 0.22 mL^{-1} (Spectronic 20 spectrophotometer). These absorbencies ensured that applied bacterial concentration was in the range of 10^7 to 10^9 CFU mL^{-1} or near 10^8 CFU mL^{-} (Bouillant et al. 1997; Miche, Bouillant, Rohr, Salle, and Bally 2000); lower bacterial concentrations showed marginal effects and gave inconsistent results. The Petri dishes were sealed with parafilm and inserted into plastic bags, wrapped in aluminium foil and incubated

upside down at 25°C. Germination and radical elongation were measured microscopically after 5 days. A seed was considered positive for germination if any radical was detectable (see Results). To relate 0.22 absorbence at 600 nm (equivalent to 0.24 at 540 nm) to the numbers of colony forming units (CFU) mL^{-1}, viable CFUs were determined for each bacterium and expressed as $\times 10^8$ CFU mL^{-1}; they were 1.7 ± 0.5 for *P. aeruginosa* QUBC1; 11.3 ± 0.6 for *P. fluorescens* QUBC3; 4.7 ± 1.0 for *M. hydrocarbonoxydans* QUBC11; 6.7 ± 0.4 for *O. anthropi* QUBC13; 0.75 ± 1.2 for *B. atrophaeus* QUBC16; 0.17 ± 0.04 for *B. subtilis* QUBC18; and 2.1 ± 0.4 for *S. epidermidis* QUBC21 (Table 2).

Identification of Orobanche *and bacterial species*

Identification of *Orobanche* spp. was based on morphological characteristics (Kreutz 1995). Bacterial identification was based on cultural, morphological, Gram stain, catalase, oxidase, and the Crystal system for diagnostic identification of bacteria (E/NF) (BBL, Becton-Dickinson and Company, Claix, France). The Crystal system was used as instructed by the supplier and the software provided. This was repeated for each tested isolate to confirm its identification.

The identities of isolates QUBC1 and QUBC2 were further confirmed using PCR according to published literature (Spilker, Coenye, Vandamme, and LiPuma 2004). Other bacteria were identified using the Universal Method (Barghouthi 2010).

Statistical analysis

Each experiment was repeated three times and contained three replicates. Pearson correlation and statistical significances were computed with the XLSTAT, (Microsoft Excel, Rosdorf, Germany), using the Tukey test after significant *F*-test ($P = 0.01$) of

Table 2. Inhibition of radical lengths of *O. cernua* or *O. aegyptiaca* seeds treated with bacteria relative to seed length and to control radicals.

		Radical length relative to seed		Radical length, % of control (% inhibition)	
Bacterium	CFU $\times 10^8 \pm$SD[1] mL^{-1}	*O. aegyptiaca*	*O. cernua*	*O. aegyptiaca*	*O. cernua*
Control (no bacteria)	0.00	3.07 ± 1.45^{a}	2.23 ± 0.82^{a}	100 (0.00)	100 (00.0)
P. fluorescens QUBC3	11.3 ± 0.6	1.07 ± 0.60^{d}	1.36 ± 0.59^{cd}	34.8 (65.2)	61.0 (39.0)
O. anthropi QUBC13	6.7 ± 0.4	1.78 ± 0.84^{c}	1.88 ± 0.66^{ab}	58.0 (42.0)	84.3 (15.7)
M. hydrocarbonoxydans QUBC11	4.7 ± 1.0	2.36 ± 0.62^{b}	1.49 ± 0.50^{bc}	76.9 (22.1)	66.8 (33.3)
S. epidermidis QUBC21	2.1 ± 0.4	1.14 ± 0.44^{d}	1.23 ± 0.32^{cd}	37.1 (62.9)	55.2 (44.8)
P. aeruginosa QUBC1	1.7 ± 0.5	0.64 ± 0.61^{ef}	1.01 ± 0.66^{cd}	20.1 (79.9)	45.3 (54.7)
B. atrophaeus QUBC16	0.75 ± 1.2	0.40 ± 0.39^{f}	0.72 ± 0.48^{d}	13.1 (86.9)	32.3 (67.7)
B. subtilis QUBC18	0.17 ± 0.04	0.93 ± 0.84^{de}	1.15 ± 0.78^{cd}	30.3 (69.7)	51.6 (48.4)

[1]Highest (top) to lowest (bottom) bacterial CFU mL^{-1} obtained at $A_{600nm} = 0.22$ mL^{-1}.

the analysis of variance (ANOVA). Means indicated with different letters are significantly different from each other.

Results

Inhibition of Orobanche aegyptiaca *Pers. radical elongation*

Amongst all tested bacterial isolates (Table 1), few bacteria (*Pseudomonas aeruginosa* QUBC1 and QUBC2, *P. fluorescens* QUBC3, *Bacillus atrophaeus* QUBC16, and *B. subtilis* QUBC18) showed significant inhibition ($P \leq 0.01$) of radical elongation of *O. aegyptiaca* relative to positive control radicals (Table 2). Bacteria that failed to inhibit radical elongation of *O. aegyptiaca* included *Stenotrophomonas maltophilia* QUBC61, *Enterobacter* sp. QUBC20, *E. cloacae* QUBC67, and *Serratia marcescens* QUBC6. Other unidentified bacterial isolates; QUBC68, SH2, SH3, SH4, SH5, L1, L2, L7, F1, and F2 were screened against *O. aegyptiaca* seeds, but failed to show any noticeable effect on the normal development of radicals. No additional work was conducted with such isolates. Investigation of *P. aeruginosa* QUBC1 effect showed a dose dependent inhibition of radical elongation (Figure 1). At low concentration, *P. aeruginosa* QUBC1 ($A_{600\text{nm}} = 0.06\text{–}4.5 \times 10^7$ CFU mL^{-1}) inhibited radical elongation of *O. aegyptiaca* by 60% and inhibition increased to 70% when bacterial concentration was increased to $A_{600\text{nm}} = 0.12\text{–}9 \times 10^7$ CFU mL^{-1}. A higher level of inhibition (79.9%) was achieved when *P. aeruginosa* QUBC1 concentration increased to $A_{600\text{nm}} = 0.22\text{–}1.7 \times 10^8$ CFU mL^{-1} (Figure 1; Table 2). High *P. aeruginosa* QUBC1 concentration reduced the average radical length to 0.64 ± 0.61 seed lengths (Ca ~0.16 mm) as compared to control radicals of 3.07 ± 1.45 and seed lengths (Ca ~0.77 mm). As illustrated in Figure 2, radical elongation of *O. aegyptiaca* was significantly ($P \leq 0.01$) inhibited by *P. aeruginosa* and other bacterial isolates; *B. atrophaeus* QUBC16, and *B. subtilis* QUBC18 when applied at high or low concentration. Although, *P. fluorescens* QUBC3 was able to significantly

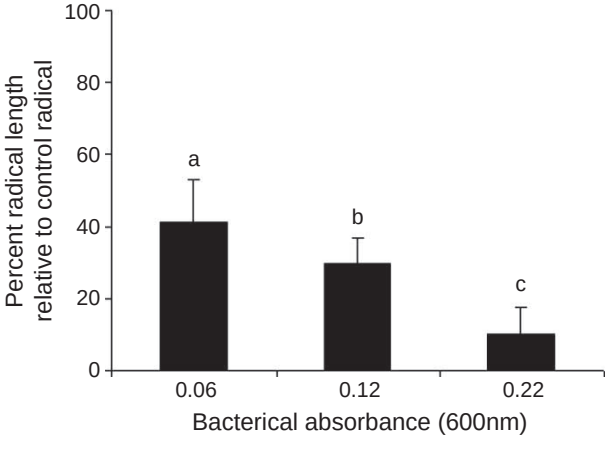

Figure 1. Effect of *Pseudomonas aeruginosa* QUBC1 concentration on radical (germ tube) length of *O. aegyptiaca*. Data with the same letters are not significantly different from each other according to Tukeys HSD test at $P \leq 0.01$ (No difference at $A_{600\text{nm}} = 0.06\ \text{mL}^{-1}$). Bars represent standard deviation (SD).

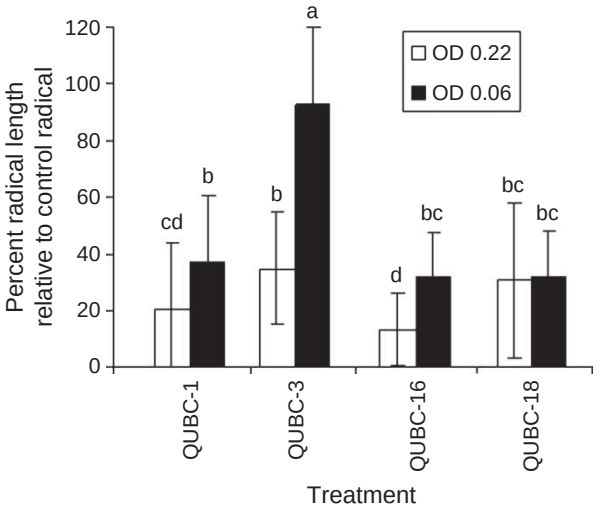

Figure 2. Efficacy of bacterial concentration ($A_{600nm} = 0.22$ and 0.06 mL^{-1}) on radical length of *O. aegyptiaca.* Tested isolates were *P. aeruginosa* QUBC1, *P. fluorescens* QUBC3, *B. atrophaeus* QUBC16, and *B. subtilis* QUBC18. Clear columns represent seed treatment with bacteria at $A_{600nm} = 0.22$ mL^{-1}. Solid columns represent seed treatment with bacteria at $A_{600nm} = 0.06$ mL^{-1}. Data with the same letters are not significantly different from each other according to Tukeys HSD test at $P \leq 0.01$. Bars represent standard deviation (SD).

($P \leq 0.01$) inhibit radical elongation of *O. aegyptiaca* when applied at high concentration (A_{600nm} of 0.22; 1.1×10^9 CFU mL^{-1}), it failed to cause significant inhibition at low concentration (A_{600nm} of 0.06; 3×10^8 CFU mL^{-1}).

The ability of different bacterial species to inhibit radical elongation was independent of the number of CFU, i.e., no correlation was found between number of CFU and the ability of a bacterium to inhibit radical elongation (Pearson, $r = 0.0004$). In Table 2, bacteria are arranged in a descending order based on CFU mL^{-1} that did not correlate with their ability to inhibit radical elongation of either species of *Orobanche.* Although *P. fluorescens* QUBC3 had the highest CFU mL^{-1} (1.1×10^9), it showed modest inhibition relative to *B. atrophaeus* QUBC16 (7.5×10^7 CFU mL^{-1}). Inhibition of radical elongation was affected by both the bacterial species and its concentration (Table 2 and Figure 2). For example, the percent of inhibition achieved by *M. hydrocarbonoxydans* QUBC11 was 22.9% while that caused by *O. anthropi* QUBC13 averaged 42% relative to normal control. These levels of inhibition were considered low relative to those obtained with *P. aeruginosa* QUBC1 (79%) or *B. atrophaus* QUBC16 (86.6%). Moderate inhibitions were obtained with *S. epidermidis* QUBC21 (62.92%) and *P. fluorescens* QUBC3 (65.23%) when applied at high concentration ($A_{600nm} = 0.22$ mL^{-1}; Table 2). Inhibition of radical elongation was significantly different when *P. aeruginosa* QUBC1, *P. fluorescens* QUBC3, or *B. atrophaus* QUBC16 bacteria were applied at lower concentration $A_{600nm} = 0.06$ mL^{-1} instead of $A_{600nm} = 0.22$ mL^{-1}. There was no significant difference ($P \leq 0.01$) between inhibition caused by low and high cell concentration *of B. subtilis* QUBC18 (Figure 2).

Inhibition of Orobanche cernua *radical elongation*

Bacterial isolates that inhibited radical elongation of *O. aegyptiaca* were targeted for testing against *O. cernua* to determine if these seeds respond in a similar fashion to the different bacteria (Figure 3; Table 2). A positive correlation was established between the ability of a bacterium to inhibit radical elongation of *O. aegyptiaca* and *O. cernua* (Pearson, $r = 0.89$). Poor correlation (Pearson, $r = 0.24$) was the observed trend between number of CFU of a bacterium and its ability to inhibit radical elongation of *O. cernua.*

Average radical length for *O. cernua* control seeds was 2.23 ± 0.82 seed lengths (Ca ~ 0.67 mm). The highest inhibitory effect (67.7%) was observed when seeds were treated with high bacterial concentration of *B. atrophaeus* QUBC16 ($A_{600\text{nm}} = 0.22$). The average radical length was reduced by 0.72 seed lengths or 0.22 mm. In addition, significant inhibition ($P \leq 0.01$) of *O. cernua* radical elongation was obtained with the other bacteria when applied at high concentration; *P. aeruginosa* QUBC1 (54.7%), *B. subtilis* QUBC18 (48.3%), *Staphylococcus epidermidis* QUBC21 (44.8%), and *P. fluorescens* QUBC3 (39%). A lower level of inhibition was scored for *M. hydrocarbonoxydans* QUBC11 (33.3%). *O. anthropi* QUBC13 failed to inhibit radical elongation (15.7%). This level of inhibition was considered as a baseline inhibition (Table 2).

Pseudomonas spp. failed to inhibit radical elongation when applied at low concentrations ($A_{600\text{nm}} = 0.06$; Figure 3). *Bacillus* spp. showed similar inhibition when applied at high or low bacterial concentration (Figure 3).

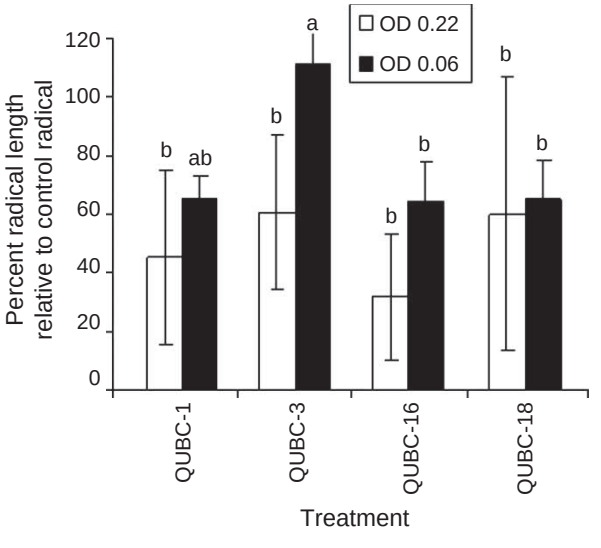

Figure 3. Efficacy of bacterial concentration ($A_{600\text{nm}} = 0.22$ and 0.06 mL^{-1}) on germ tube length of *O. cernua*. Tested isolates were *P. aeruginosa* QUBC1, *P. fluorescens* QUBC3, *B. atrophaeus* QUBC16, and *B. subtilis* QUBC18. Clear columns represent seed treatment with bacteria at $A_{600\text{nm}} = 0.22$ mL^{-1} and solid columns represent seeds treated with bacteria at $A_{600\text{nm}} = 0.06$ mL^{-1}. Data with the same letters are not significantly different from each other according to Tukeys HSD test at $P \leq 0.01$. Bars represent standard deviation (SD).

Discussion

Several bacterial genera exhibit biocontrol activity (Chernin, Brandis, Ismailov, and Chet 1996; Raaijmakers, Vlami, and de Souza 2002); Studies conducted by Ahonsi, Berner, Emechebe, and Lagoke (2002) focused on the ability of different *Pseudomona fluorescens/putida* strains to control *Striga* in pot experiments. Zermane et al. (2007) working with *P. fluorescens* Bf7-9 in pot experiments showed that the bacterium was able to inhibit the growth of both *Orobanche crenata* Forsk. and *O. foetida* Poir.

The two isolates of *P. fluorescens* (WSM3455 and WSM3456) obtained after screening 442 strains, together with *Alcaligenes xylosoxidans* (WSM3457) can inhibit wild radish without any significant deleterious effects on either grapevine rootlings or subterranean clover. *P. fluorescens* (WSM3455) produces hydrogen cyanide (Vargas and O'Hara 2006) which is involved in the breaking of seed dormancy (Oracz et al. 2009). Biocontrol activities against plant-pathogenic fungi by various

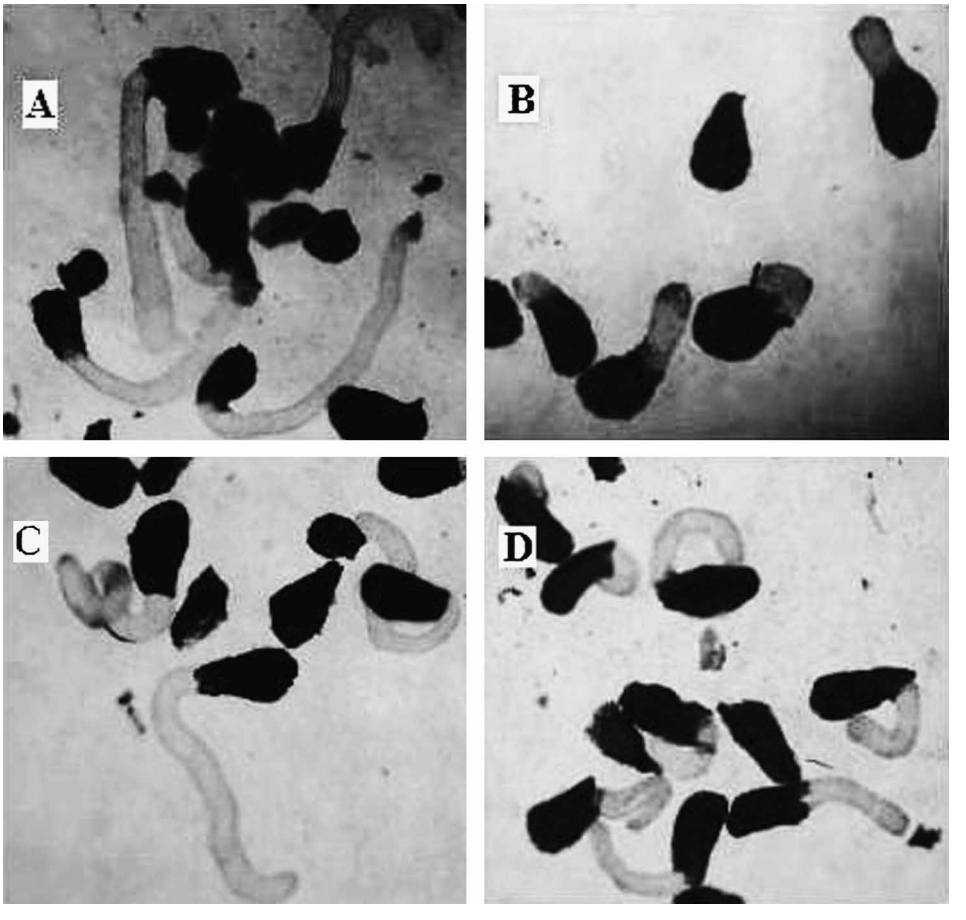

Figure 4. Radical lengths of *Orobanche* spp. as observed with low power microscopy (4 ×) in presence or absence of bacteria ($A_{600nm} = 0.22$ mL^{-1}). (A) control untreated *O. aegyptiaca* seeds; (B) control untreated *O. cernua* seeds; (C) *O. aegyptiaca* and (D) *O. cernua* seeds treated with *P. aeruginosa* QUBC1 $A_{600nm} = 0.22$ mL^{-1}.

fluorescent pseudomonads are well known (Ovadis et al. 2004). Strategic approach to weed biocontrol include *P. syringae* pv. *tagetis* as a broad-spectrum pathogen (Charudattan 2001). The *in vitro* results presented in this work with *P. fluorescens* QUBC3 are in agreement with published literature. *P. fluorescens* QUBC3 inhibited radical elongation of both *O. aegyptiaca* and *O. cernua*. when applied at high concentration ($A_{600nm} = 0.22–1.1 \times 10^9$ CFU mL^{-1}).

Phenolic compounds concentrate at the apices of elongation-inhibited radicals (Nun, Dadon, and Mayer 2005). *P. fluorescens* NCIB 3756 and *P. aeruginosa* NCIB 950 are known to biodegrade phenols (Agarry et al. 2008) suggesting a role in the ability of *P. aeruginosa* to promote plant growth (Mansoor, Sultana, and Haque 2007). Injured pea roots are prompted to produce two phenolic compounds, gallic acid and naringenin, which inhibit GR24 induced germination of *O. crenata* (Mabrouk et al. 2007a,b). Although the ability of *P. aeruginosa* QUBC1 to inhibit radical elongation can not be directly linked to its ability to degrade phenols, the bacterium may degrade intracellularly or extracellularly, or chemically modify GR24. Alternatively, it may produce strigolactone-like hormones that act to inhibit radical elongation, or produce GR24 inhibitors. It is not clear yet whether bacterial attachment or culture supernatant is responsible for the observed inhibition.

Reports on the application of *Bacillus* spp. as biocontrol agents of weeds are not common; however *B. subtilis* produces a number of antibacterial antibiotics and antifungal compounds including lipopeptides (Leclère et al. 2005). In this study, highest mean inhibition of radical elongation (86.9% for *O. aegyptiaca* and 67.7% for *O. cernua*) was recorded with *B. atrophaeus* QUBC16 (Figure 3; Table 2). The isolates *B. atrophaeus* QUBC16 and *B. subtilis* QUBC18 had significantly ($P \leq 0.01$) inhibited radical elongation of both *Orobanche* spp. when applied at high or low concentration (Figures 2 and 3; Table 2).

Inhibition of germination and radical growth of *O. aegyptiaca* is thought to be mediated by a small alcohol soluble peptide ($\leq$1000 Da) produced by *Azospirillum brazilense*. Competitive inhibition to the germination receptor is probably responsible for the peptide effect (Nun et al. 2005). *A. brasilense* does inhibit *Striga* seed germination as well (Bouillant et al. 1997, Miche et al. 2000). Lipophilic compounds extracted from log and stationary growth culture media prevent the germination of *Striga* seeds, whereas high concentrations of *A. brasilense* (10^{10} CFU mL^{-1}) failed to block seed germination in presence of GR24 (Miche et al. 2000).

Since reported biocontrol bacterial species are inclined to inhibit radical elongation rather than seed germination (this work, Miche et al. 2000), our current investigation is focusing on several important areas. First, we are searching for bacteria that will stimulate seed germination in the absence of host plant or GR24. Second, it is important to find bacteria that will colonise and destroy weed seeds before they germinate. Both activities, stimulation of germination and destruction of viable seeds, can be assayed when a bacterium is applied to *Orobanche* sp. seeds in the absence of GR24 or host plant. The possible outcomes of such a test are stimulation of seed germination, reduction of viable seeds, or no effect. Bacteria may control weeds by interrupting signals required for germination, radical elongation, haustorium formation, rhizotropism, or attachment. Identification of the factors that may modulate bacterial inhibition of radical elongation will allow selection of bacterial isolates with promise as weed biocontrol agents. Identification of these factors and their effects will render controlling weed growth possible. Special effort

will be devoted to understanding the mechanism(s) of inhibition caused by *B. atrophaeus* and *P. aeruginosa*. Currently, several variables such as dipicolinic acid, spore formation, culture supernatants, bacterial cell extracts, bacterial mutants, and other factors are being investigated.

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