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Biological Control of Watermelon Seedling Blight Caused by *Acidovorax citrulli* Using Antagonistic Bacteria from the Genera *Curtobacterium*, *Microbacterium* and *Pseudomonas*

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Abstract

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The biological control of the watermelon seedling blight and fruit blotch disease was investigated by screening the potential use of antagonistic bacteria. Between May and August 2012, totally 322 putative antagonistic bacteria were isolated from symptomless melon and watermelon plants grown in Adana, Hatay, and Osmaniye provinces of the Eastern Mediterranean Region of Turkey. *In vitro* dual culture tests showed that 54 out of 322 strains inhibited the *Acidovorax citrulli* (Ac) growth with an appearance of clear zones between 2.3 and 27.0 mm in diameter. However, the remaining 268 strains did not exhibit any antagonistic activity against Ac. Seed treatments with fourteen individual antagonistic bacteria resulted in a significant reduction in disease incidence (DI) and severity (DS) ranging between 14.06–79.47% and between 4.57–41.49%, respectively. The bacteria *Pseudomonas oryzihabitans* (Antg-12), *Microbacterium oxydans* (Antg-57), *Curtobacterium flaccumfaciens* (Antg-198), and *Pseudomonas fluorescens* (Antg-273) were the most potent antagonistic bacterial isolates which reduced DI and DS as compared to the untreated control. This study suggested the potential of bacterial antagonists *Curtobacterium flaccumfaciens, Microbacterium oxydans, Pseudomonas oryzihabitans*, and *Pseudomonas fluorescens* for the biocontrol of Ac-induced bacterial fruit blotch (BFB).

Keywords: *Acidovorax citrulli*; cucurbist; biocontrol; seed treatment

Bacterial fruit blotch (BFB) disease caused by the bacterium *Acidovorax citrulli* Schaad *et al*. 2009 (formerly *A. avenae* subsp. *citrulli*; Schaad *et al.* 1978; Willems *et al.* 1992) is a devastating disease of cucurbits and poses a great threat threat to cucurbit seed and fruit production throughout the world (Burdman *et al.* 2005; Walcott 2005; Mirik *et al.* 2006; Ren *et al.* 2006; Feng *et al.* 2013). Apart from fruit blotch, *A. citrulli* also causes leaf and seedling blight and blossom rot in cucurbitaceous plants (Frankle *et al.* 1993; Lessl *et al.* 2007; Wang *et al.* 2009). The symptoms of BFB include water-soaked, greasy or irregular brown spots on true leaves which are not so characteristic. However, disease symptoms on the fruit appear as water-soaked, dark and olive-green stain or blotch lesions with irregular margins that are rapidly enlarged with brown discoloration and brown cracks on the surface within 10 days after bacterial contamination. In the mesocarp of the infected fruits, water-soaked and smooth spots also occur and these rottings make the fruits unmarketable (Latin & Hopkins 1995).

BFB is a seed transmittable disease (O'Brien & Martin 1999), because the bacterium can survive on/in seeds over 30 years under suitable storage conditions (Block & Shepherd 2008). Thus, seed

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treatments and using bacteria-free seeds must be the primary measure for the disease control (Melo *et al.* 2014). Many attempts including chemical and physical seed treatments have been developed to get rid of seedborne *A. citrulli*. Chemical and biochemical treatments and fermentation of cucurbitaceous seeds with chitosan, streptomycin sulphate, sodium hypochlorite, peroxyacetic acid, mercury chloride, hydrochloric acid, calcium chloride, copper-containing bactericides reduced the incidence of the disease. However, since the pathogen can be localised under the seed coat (Rane & Latin 1992), the chemicals failed to eliminate the pathogen from seeds (Sowell & Schaad 1979; Rane & Latin 1992; Hopkins *et al.* 1996, 2003; Lovic & Hopkins 2003; Zhao *et al.* 2003; Feng *et al.* 2007; Selcuk 2014). Other alternative disease management strategies such as physical seed treatments were employed alone or combined with chemicals and they all varied widely in efficacy, but they did not completely remove the pathogen from the seeds (Nomura & Shirakawa 2001; Hopkins & Thompson 2006).

Even though early reports were successful in reporting some promising resistance genes in cucurbit cultivars (Goth & Webb 1981; Hopkins & Thompson 2002) and the authors could identify several tolerant lines in melon, however, there are no available BFB-resistant cultivars (Bahar *et al.* 2009). The identification of resistance is quite complicated, since *A. citrulli* can infect in all growth stages of cucurbits and the bacterial strains and monitored plant stage may influence the level of resistance (Johnson *et al.* 2011). In the absence of resistance in watermelon, there is a need to use other environmentally safe components that could be integrated into a disease management strategy of BFB on watermelon.

Biological control with antagonistic bacteria or yeasts has been considered to be a friendly and useful approach for plant disease management including BFB on cucurbits (Fessehaie & Walcott 2005; Medeiros *et al.* 2009; Wang *et al.* 2009; Jiang *et al.* 2015; Melo *et al.* 2015). Fessehaie and Walcott (2005) reported that when the maize pathogenic bacteria *Acidovorax avenae* subsp. *avenae* AAA99-2 or *Pseudomonas fluorescens* A506 were used as seed or blossom treatment in watermelon, bacterial blotch transmission to seedlings or seed infestation was highly reduced under greenhouse conditions. In that study, 96 strains of epiphytic and endophytic bacteria isolated from symptomless melon plants were tested as seed treatment for the biocontrol of BFB under greenhouse conditions. The authors demonstrated that microbiolisation of artificially infested seeds with *Bacillus* sp. yielded 93.7% disease severity reduction (Oliveira *et al.* 2006). Some other scientists also reported the potential use of *Bacillus* sp. in reducing the incidence and severity of BFB (Santos *et al.* 2006; Medeiros *et al.* 2009; Jiang *et al.* 2015). The studies in the biocontrol of BFB with antagonistic bacteria as seed treatment have a potential to get rid of unmarketable seedlings or fruits. Since there is no available biological control product in the market for BFB management, more researches should be conducted.

The main objectives of this study were (*i*) to isolate candidate antagonistic bacteria for the biological control of BFB, (*ii*) to screen the *in vitro* antibiosis ability of biocontrol agents, (*iii*) to evaluate the efficacy of antagonistic bacteria in the biological control of BFB via seed treatment, and (*iv*) to identify the most potent antagonistic bacteria.

MATERIAL AND METHODS

Pathogenic bacterium, media and preparation of bacterial inoculum. The pathogen Tasci-1 of *Acidovorax citrulli* (Ac) obtained from the culture collection of the Laboratory of Phytobacteriology (Cukurova University, Faculty of Agriculture, Department of Plant Protection, Adana, Turkey) was isolated from a naturally contaminated watermelon fruit grown in Cukurova Region, Turkey in 2009. The pathogenic bacterium was identified using WFB1/WFB2 primer pairs (Walcott & Gitaitis 2000). King's Medium B (KB) (Lelliott & Stead 1987) was used for culturing the pathogen in Petri dishes. To prepare *A. citrulli* inoculum from a routinely grown culture on KB at 25°C for 48 h, a suspension was prepared in saline buffer (NaCl 0.85%) and the concentration was adjusted to A_{600} 0.2 OD with a spectrophotometer (UV-120-01; Shimadzu, Kyoto, Japan). The bacterial suspension was 10-fold serially diluted with saline buffer and aliquots of 100 µl were inoculated onto KB. The concentration of the bacterial inoculum was estimated to be 1.4×10^8 CFU/ml and this concentration was used in further studies.

Isolation of antagonists and culture preparation. Between May and August 2012, antagonistic bacterial strains were isolated from symptomless melon and watermelon plants grown in crop fields of Adana, Hatay, and Osmaniye Provinces of the Eastern Medi-

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terranean Region of Turkey. The symptomless and fresh leaves and blossoms were used for isolations. Each field was surveyed and samples were collected randomly to get a homogeneous collection. Soil samples were collected from healthy plants to receive beneficial root-colonising bacteria. Sampled blossoms were used directly, whereas leaves were cut into small pieces, and soil samples were sieved to avoid big fragments. About 10 g of each sample were placed in Erlenmeyer flasks containing 90 ml of nutrient broth (NB) and shaken on a rotary shaker (Model Unimax 1010; Heidolph Instruments, Schwabach, Germany) at 250 rpm at 25°C for 2 hours. The mixture in each flask was filtered through single-layered cheesecloth. One volume of each suspension was diluted and designated as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} in a serial dilution of 9 ml saline buffer and aliquots of 100 µl were pipetted onto 90 mm Petri dishes containing KB, nutrient yeast dextrose agar (NYDA) (Droby *et al.* 1990) or National Botanical Research Institute's phosphate growth (NBRIP) (Nautiyal 1999) media. The suspension drop of each dish was homogenised onto the medium using a flame-sterilised glass rod. All plates were incubated at 25°C for 48 hours. The colonies were individually separated according to morphological features including colour, appearance and colony type. Candidate bacterial antagonists were labelled and transferred to new glass tubes with yeast extract calcium carbonate agar (YDCA) medium and stored at –20°C until use.

In-vitro antibiosis screening of bacterial strains against Ac. Putative antagonistic bacteria were tested for their ability to suppress Ac growth using the dual culture test described by Krishnamurthy and Gnanamarickam (1998). The two-days-old cultures were used for *in vitro* screenings of bacterial strains. Briefly, candidates were individually spotted onto the middle of KB medium and Petri dishes were incubated at 25°C for 48 hours. Three replicates were used for each strain. After 48 h, suspensions of Tasci-1 Ac (adjusted to 1.4×10^6 CFU/ml) were sprayed onto the plates and were additionally incubated at 25°C for 48 hours. The appearance of clear zones was determined as the inhibition of Ac growth and zones were measured. The inhibition ability of antagonists was rated using a 0–6 scale (0 = no zones, 1 = 1–4 mm zone, 2 = 5–8 mm zone, 3 = 9–12 mm zone, 4 = 13–16 mm zone, 5 = 17–20 mm zone, and 6 = ≥ 21 mm zone).

Efficacy of bacterial antagonists as seed treatments. Watermelon seeds cv. Crimson Sweet (Nickerson Zwaan, Made, The Netherlands) were obtained as untreated with chemicals. The seeds were immersed into 200 ml of 1% carboxymethyl cellulose (C5678; Sigma Aldrich, Taufkirchen, Germany) and Tasci-1 of *A. citrulli* suspension (1.4×10^6 CFU/ml) for 30 min and filtered through double-layered cheesecloth to get rid of bacterial residues. Seeds were dried at room temperature (25 ± 2°C) for 24 h (Wang *et al.* 2009) and divided into three lots (33 seeds/lot). Pathogen-treated watermelon seed lots were kept in paper bags in the fridge at 4°C for 3–7 days until use.

For the preparation of antagonistic bacterial suspensions, bacterial cells were suspended in 9 ml of saline buffer and the concentration of cells in each culture was adjusted to A_{600}: 0.2 OD using a spectrophotometer. Seed lots were immersed into antagonist suspensions for 30 min, dried overnight and each lot was sown in plastic pots (17 × 28 cm, height × diameter) containing sterile soil. The pots were kept under climate room conditions with the following conditions: 30–32°C, 60% humidity, and 16 h of light alternating with 8 h of darkness. Ten days after seed germinations, each cotyledon was examined for blight symptoms. For BFB seedling transmission, DI (number of seedlings displaying typical BFB symptoms divided by the number of seedlings that germinated × 100) was recorded.

A modified 0–7 score (0 = symptomless cotyledons; 1–7 = cotyledon blights covering 1–10, 11–20, 21–35, 36–50, 51–70, 71–85, and 86–100%, respectively), in which 7 indicates damping off of cotyledons, was used for DS (Araujo *et al.* 2005; Amadi *et al.* 2009). DS and biocontrol efficacy were calculated as follows:

Disease severity (%) = [(Σ number of diseased leaves in each grade × grade)/(total number of leaves invesigated × the highest disease inde)] × 100

Biocontrol efficacy (%) = [(incidence rate in the control – incidence rate in the antagonist – trated group)/incidence rate in the control] × 100

The experimental design was completely randomised with three replicates, and each replicate consisted of thirty-three seedlings. The experiment was repeated twice in controlled conditions.

Characterisation of bacterial antagonists. The antagonistic bacteria were identified according to morphological and molecular features. After the incubation on KB for 48 h, the size, colour, and appearance of bacteria were recorded. The bacterial

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strains were sequenced using 27F [5'-AGAGTTTGATCMTGGCTCAG-3'] and 1492R [5'-TACGGYTACCTTGTTACGACTT-3'] primers based on the region identity within 16S rRNA (Lane 1991). The PCR products were examined on 1% agarose gel. The sequences were compared by using the basic local alignment search tool (BLAST) with the reference sequences in the NCBI (National Centre for Biotechnology Information).

Data analysis. Data on diameter of inhibition zones, disease incidence and disease index for the antagonistic bacterial strains were analysed using analysis of variance (ANOVA) in CoStat v6.4 statistics software (CoHort Software, Pacific Grove, USA). Data on the mean inhibition zones were arcsine-transformed to angular data before ANOVA. The % efficacy of bacterial antagonists was calculated according to Abbott's Formula: [(the number of cotyledons with blight symptoms in control treatment – the number of cotyledons with blight symptoms in bacterial antagonist treatment)/the number of cotyledons with blight symptoms in control] × 100.

RESULTS

Isolation of antagonists and culture preparation. A total of 322 candidate antagonists were isolated from twenty-eight and nine individual watermelon and melon crop fields (Adana, Hatay, and Osmaniye Provinces) in the Eastern Mediterranean Region of Turkey. Among 37 individual watermelon and melon fields, 31, 23, and 37 leaves, blossoms and soil samples were collected, respectively (Table 1).

In-vitro antibiosis screening of bacterial strains against Ac. A total of 322 candidate antagonistic bacterial strains were screened for their potential of inhibiting Ac growth in petri dishes using the dual culture test. Results of *in vitro* screening showed that 54 out of the 322 strains tested on KB medium inhibited Ac growth, indicating the appearance of clear zones in the dishes. The remaining 268 strains were not antagonistic to Ac. The average of clear zones for 54 antagonistic strains varied between 2.3 mm to 27.0 mm in diameter. According to the 0–6 scale, out of 54 antagonists, 4, 10, 12, 17, 7, and 4 were placed in 1 to 6 rating scores, respectively. There was a significant difference ($P \leq 0.05$) in the mean diameter of clear zones between 54 antagonistic bacteria. Then, 14 individual strains were chosen according to morphological features and different statistical groups for seed treatments. The inhibition ability of selected antagonists ranged from 6.4 mm to 27.0 mm (Figure 1). In the *in vitro* antibiosis tests, four of the selected biocontrol agents (Antg-189 – 27.0 mm, Antg-147 – 22.1 mm, Antg-57 – 21.5 mm, and Antg-144 – 21.3 mm) significantly inhibited the pathogen growth in the dishes (Figure 1).

Fourteen bacterial antagonists were characterised according to morphological and molecular aspects. Colonies of bacterial strains were cream, yellow, orange, and white. Based on molecular analyses, they were characterised as *Pseudomonas oryzihabitans* (Antg-12 and Antg-61), *Microbacterium oxydans* (Antg-57 and Antg-79), *Bacillus methyllotrophicus* (Antg-97), *Paenibacillus jamilae (*Antg-101), *Paenibacillus polymyxa* (Antg-144), *Proteus mirabilis* (Antg-147), *Curtobacterium flaccumfaciens* (Antg-189 and Antg-198), *Pseudomonas parafulva* (Antg-197), *Pseudomonas putida* (Antg-223 and Antg-262), and *Pseudomonas fluorescens* (Antg-273). All tested antagonists were placed in different groups on a phylogenetically constructed tree using UPGMA.

Table 1. Bacterial strains isolated from Adana, Osmaniye, and Hatay Provinces

Plant samples	Adana	Osmaniye	Hatay	Total number of strains
Leaves	70	32	–	102
Blossoms	81	23	–	104
Soils	80	32	4	116
Total	231	87	4	322

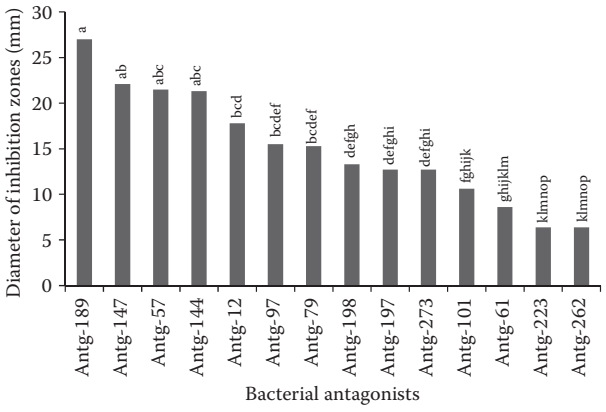

Figure 1. Growth inhibition of *Acidovorax citrulli* (Ac) using different bacterial antagonists on KB in a dual culture test

Bars headed with the same letters are not significantly different ($P \leq 0.05$) according to Duncan's Multiple Range Test

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Table 2. Efficacy of different bacterial antagonists as seed treatments evaluated by disease incidence and disease severity (means of two experiments with 3 replicates of all 33 seedlings)

Bacterial antagonists	Disease incidence (%)	Efficacy (%)	Disease severity (%)
Control	92.40[a]		64.35
Pseudomonas oryzihabitans (Antg-61)	79.47[b]	13.98	36.53
Curtobacterium flaccumfaciens (Antg-189)	75.25[b]	18.82	41.49
Bacillus methylotrophicus (Antg-97)	61.25[c]	33.79	28.77
Paenibacillus jamilae (Antg-144)	59.33[c]	35.88	30.37
Paenibacillus polymyxa (Antg-101)	52.93[c]	42.91	24.59
Pseudomonas putida (Antg-262)	48.10[cd]	47.56	22.29
Pseudomonas putida (Antg-223)	45.55[cd]	50.78	23.73
Proteus mirabilis (Antg-147)	37.48[de]	58.70	12.52
Microbacterium oxydans (Antg-79)	28.41[ef]	68.75	10.23
Pseudomonas parafulva (Antg-197)	23.51[ef]	74.06	7.21
Microbacterium oxydans (Antg-57)	20.55[f]	77.40	6.02
Pseudomonas fluorescens (Antg-273)	16.80[f]	81.55	9.81
Pseudomonas oryzihabitans (Antg-12)	16.22[f]	82.12	4.57
Curtobacterium flaccumfaciens (Antg-198)	14.06[f]	84.73	5.30

Means followed by the same letters within each column are not significantly different ($P \leq 0.05$) according to Duncan's Multiple Range Test

Efficacy of bacterial antagonists as seed treatments. Treatment of watermelon seeds with Ac alone resulted in development of seedling blights with severe symptoms on cotyledons. Since the results obtained from the first and second experiment were compatible, the means of both trials were analysed. The mean disease incidence and disease severity were 92.40 and 64.35% in control plants. However, DI and DS in bacterial antagonist-treated seedlings were 79.47–14.06 and 36.53–5.30%, respectively. All of the tested antagonists significantly reduced ($P \leq 0.05$) the DI and DS in trials (Table 2). *Pseudomonas oryzihabitans* (Antg-12), *Microbacterium oxydans* (Antg-57), *Curtobacterium flaccumfaciens* (Antg-198), and *Pseudomonas fluorescens* (Antg-273) highly reduced DI and DS as compared with the untreated control (Table 2) in reducing watermelon seedling blight caused by *Acidovorax citrulli* (Ac). None of the treatments with antagonistic bacteria adversely affected the seed germination.

DISCUSSION

Biological control of various plant diseases using microorganisms is an effective approach to control diseases in an eco-friendly manner. The first step is to screen for potential biological control agents, and the main screening method under *in vitro* conditions is based on antagonistic activity. Current findings confirmed a strong inhibitory activity of 40 individual bacterial antagonists on BFB growth. These results are consistent with previous reports (Fessehaie &Walcott 2005; Medeiros *et al.* 2009; Jiang *et al.* 2015) and indicated that the bacterial pathogen growth was inhibited under *in vitro* conditions.

BFB is a seedborne bacterial disease (Sowell & Schaad 1979) causing serious economic losses. Therefore, many attempts including seed treatments were made to control the disease. Physical and chemical treatment of seeds was recommended for controlling BFB (Sowell & Schaad 1979; Lovic & Hopkins 2003; Burdman & Walcott 2012; Mengulluoglu & Soylu 2012), however, some of those treatments adversely affected seed germination (Hopkins *et al.* 2003; Hopkins & Thompson 2006). In our study, biological control agents were efficient in reducing disease severity and disease index compared to other seed treatment methods. None of the antagonistic microorganisms influenced seed germination or quality parameters of seeds (Walcott 2005; Medeiros *et al.* 2009; Wang *et al.* 2009; Johnson *et al.* 2011). Neither did the tested bacterial antagonists affect seed germination in this study. Therefore, it was

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suggested that biological seed treatments should be included in the BFB disease management programs.

In the BFB cycle, seeds are the primary inoculum sources for the infection of the plant (Latin & Hopkins 1995) and infested seed can readily introduce BFB epidemics in commercial greenhouses and fields under favourable conditions. The use of bacteria-free seeds is the primary measure recommended for disease control (Latin & Hopkins 1995). In seed lots, one seed infested with Ac has the potential to spread in the greenhouse.

To date, seed treatments were suggested to reduce or eliminate seed contamination, however, no treatment is 100% effective against the bacterial agent. Burdman and Walcott (2012) explained that the effectiveness of seed treatments could be influenced by penetration to the seed coat and by the localisation of the bacteria in/on seeds. Use of naturally or artificially contaminated seeds in researches also influences the efficacy of seed treatments. Rane and Latin (1992) treated naturally infected or experimentally inoculated watermelon seeds with hot water, NaOCl, and HCl. The amount of infection ranged from 7% of naturally infected Prince Charles fruit to nearly 96% of artificially inoculated seeds. All treatments significantly reduced the pathogen in seedlings that developed from naturally or artificially contaminated seeds, but no treatment completely eliminated the pathogen within the seeds. The effect of seed treatments on disease emergence was reduced by 69–74 and 88–91% in seeds naturally or artificially infested with the pathogen. In present findings, biological control agents inhibited the pathogen growth reaching 10–94%. These results can be explained by the pathogen localisation in the seed and storage period of treated seeds. Previously, it was hypothesised that Ac cells may be localised in the testa layer in artificially treated seeds. The testa was not a major site of Ac accumulation in infested seeds. Ac was detected at low percentages in the testae compared with those in embryo and endosperm layer samples. When the testae were removed from the pericarp and pistil of inoculated seeds, BFB seedling transmission was not significantly affected. These findings indicated that Ac cells that accumulated in the testae may not be epidemiologically significant, especially for long-time stored seeds (Dutta *et al.* 2012). Latin and Rane (1992) demonstrated seed transmission from both naturally diseased and artificially inoculated symptomatic fruit. The recovery of the pathogen from the testae and embryos of seeds indicated the contamination of seeds externally and internally. In naturally infected seeds, the bacterium can be localised externally and internally, however, externally applied seed treatments can vary in artificial inoculations. That is why the use of naturally contaminated seeds enhances the disease control capacity of the various seed treatments. In this study, watermelon seeds were artificially inoculated since the pathogen is a quarantine organism and it is impossible to find naturally infested seeds from producers.

Many antagonistic bacteria from different genera such as *Bacillus, Paenibacillus* and *Pseudomonas* and yeasts were identified to be efficient in controlling BFB (Fessehaie & Walcott 2005; Oliveira *et al.* 2006; Medeiros *et al.* 2009; Wang *et al.* 2009; Conceição *et al.* 2014; Jiang *et al.* 2015; Melo *et al.* 2015). In this study, the identified antagonists *Pseudomonas oryzihabitans* and *Pseudomonas fluorescens* extremely reduced the disease development up to 82% in antagonist treated seeds. While the *Pseudomonas putida* strains showed a greater disease control of BFB up to 65%, the antagonists *Paenibacillus polymyxa* and *P. jamilae* inhibited the pathogen growth over 50%. Kheirandish and Harighi (2015) evaluated the antagonistic effects of some rhizobacteria against *Ralstonia solanacearum*. Seven isolates with inhibitory effects on the pathogen were identified as *Pseudomonas putida, Paenibacillus* sp., and *Serratia* sp. and antagonists significantly reduced the disease by 38–56%. *Curtobacterium* sp. and *Microbacterium oxydans* had a potential to control pests (Ozsahin *et al*. 2014) and parasitic nematodes (Aballay *et al*. 2012). PGPR treatments with *Curtobacterium flaccumfaciens* strain ME1 reduced the severity of foliar diseases caused by *Pseudomonas syringae* pv. *lachrymans* and *Colletotrichum orbiculare* both in greenhouses and fields (Raupach & Kloepper 1998, 2000). Since *Curtobacterium flaccumfaciens* has been identified as a potential antagonist for a plant bacterial disease, no study has reported the disease control of BFB in cucurbits. Although *Microbacterium* sp. was isolated from the grape fruit surface (Kántor & Kačaniová 2015), maize rhizosphere (Gao *et al*. 2013), and foreshore soils (Irshad *et al*. 2013), *Microbacterium oxydans* has not yet been identified as a biocontrol agent for plant diseases, not even for BFB. To the best of our knowledge, the present study is the first approach indicating that *Curtobacterium flaccumfaciens* and *Microbacterium oxydans* have a high potential usage as seed treatments in the biological control of BFB. As known, no biopesti-

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cide is available for BFB, our works will continue to commercialise those promising antagonists to be effectively used as seed treatments in nurseries.

Acidovorax citrulli, the agent of seedling blight and fruit blotch of cucurbits, can affect several plant organs during different stages of development. Therefore, the selection of biological control agents for the disease control should consider different disease stages to get more reliable results. The bacteria *Curtobacterium flaccumfaciens, Microbacterium oxydans, Pseudomonas oryzihabitans*, and *Pseudomonas fluorescens* were highly efficient for treating watermelon seeds. Thus, the application of these antagonistic bacteria should be combined with other control strategies such as resistant cultivars, disinfection or fermentation of seeds, spray of copper compounds. Those antagonists should be applied under greenhouse or field conditions to compare their efficacy on BFB development.

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