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Control of damping-off of organic and conventional cucumber with extracts from a plant-associated bacterium rivals a seed treatment pesticide

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ABSTRACT

Environmentally friendly control measures are needed for soilborne diseases of crops grown in organic and conventional production systems. We tested ethanol extracts from cultures of *Serratia marcescens* N4-5 and N2-4, *Burkholderia cepacia* BC-1 and BC-2, and *Burkholderia ambifaria* BC-F for control of damping-off of cucumber caused by the soilborne pathogens *Pythium ultimum* and *Rhizoctonia solani*; ethanol being an Organic Materials Review Institute (OMRI) -approved solvent for use in certain applications in organic crop production. Ethanol extracts from strains N4-5 and N2-4 inhibited mycelial growth and germination of sporangia of *P. ultimum in vitro* but those from strains BC-1, BC-2, BC-F, and the ethanol control did not. Ethanol extracts from strains BC-2 and BC-F inhibited mycelial growth of *R. solani in vitro* while ethanol extracts from strains BC-1, N2-4, N4-5, and the ethanol control did not. Thin-layer chromatography demonstrated that ethanol extracts from strain N4-5 contained prodigiosin while ethanol extracts from strains BC-2 and BC-F contained pyrrolnitrin; extracts from strains N2-4 and BC-1 did not contain either of these compounds. DNA sequencing confirmed the presence of a biosynthetic gene for prodigiosin in strain N4-5 and its absence in strain N2-4, while a biosynthetic gene for pyrrolnitrin was found in strains BC-2 and BC-F but not in strains N2-4, N4-5, and BC-1. Prodigiosin was previously implicated in inhibition of *P. ultimum* while pyrrolnitrin has been shown to inhibit *R. solani*. Certified-organic cucumber seed treated with an ethanol extract of strain N4-5 was the only extract treatment from any of these five microbial strains to effectively suppress damping-off caused by *P. ultimum* in growth chamber pot experiments. This ethanol extract provided suppression of *P. ultimum* on cucumber that was similar to that provided by a commercially available seed treatment pesticide and greater than that provided by a commercially available biocontrol agent for this pathogen. The inhibitory factor(s) in ethanol extracts of strain N4-5 was stable as a seed treatment for at least 14 weeks when incubated at 4 °C in the dark. No ethanol extracts applied as treatments of organic cucumber seed consistently suppressed damping-off caused by *R. solani* in growth chamber pot experiments. Experiments reported here suggest that certain natural products from microbial strains as seed treatments are promising alternatives for control of soilborne diseases in conventional or organic cucumber production systems.

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1. Introduction

Soilborne plant pathogens can cause major reductions in crop yields (Raaijmakers et al., 2009). Development of new control measures for use in conventional crop production systems for soilborne pathogens are needed due to problems associated with the availability and effectiveness of chemical controls and host resistance. With regard to chemical controls, increased public

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Table 1
Microbial strains and primers.

Microbial isolate or primer	Relevant characteristics	Source, prior designation, and/or reference
Bacterial isolate		
Burkholderia cepacia BC-1	Suppresses *Pythium ultimum* and *Rhizoctonia solani*	W. Mao; Roberts et al. (2005)
B. cepacia BC-2	Produces pyrrolnitrin; suppresses *P. ultimum*	W. Mao; Roberts et al. (2005); this study
B. ambifaria BC-F	Produces pyrrolnitrin; suppresses *P. ultimum*	W. Mao; Roberts et al. (2005); this study
Pseudomonas protegens Pf-5	Formerly *P. fluorescens* Pf-5; positive control for pyrrolnitrin production	de Souza and Raaijmakers (2003)
Serratia marcescens ATCC 274	Positive control for prodigiosin and serrawettin W1 production	Roberts et al. (2007)
S. marcescens ATCC 8100	Negative control for prodigiosin and serrawettin W1 production	Roberts et al. (2007).
S. marcescens N2-4	Nonpigmented isolate of *S. marcescens*; suppresses *P. ultimum*	D. Kobayashi; Roberts et al. (2005)
S. marcescens N4-5	Produces prodigiosin; suppresses *P. ultimum*	Roberts et al. (2007)
Fungal/Oomycete isolate:		
Pythium ultimum Puzc	Pathogenic on cucumber	Roberts et al. (2005, 2007)
Rhizoctonia solani Rs-23A	AG-4; pathogenic on cucumber	Rs23A; Lakshman et al. (2012); Lewis and Papavizas (1987); Roberts et al. (2005)
Primer:		
PIGF1	ATT GCA AAA TCG CAT CAA GG	Roberts et al. (2007)
PIG-R1	AGA ACC AGG TTT CGG TGA CG	
PRND1f	GGG GCG GGC CGT GGT GAT GGA	This study
PRND2r	YCC CGC SGC CTG YCT GGT CTG	
SW2–F3	GCG ACA AAA GCA ATG ACA AA	Roberts et al. (2007)
SW2-R3	GTC GGC GTA TTG TTC CAA CT	

awareness concerning the hazards associated with their use has led to a more stringent regulatory environment; resulting in the ban of widely used chemical controls, such as methyl bromide, and increased difficulty in getting new pesticides on the market (Ruzo, 2006; Zasada et al., 2010; Glare et al., 2012). Further compounding the problem is the occurrence of fungicide resistance in pathogens, and the lack of, or breakdown of, host resistance to soilborne pathogens (Martin, 2003; Bonanomi et al., 2007; Louws et al., 2010). Organic production systems also need additional control methods for soilborne pathogens as these production systems rely largely on host resistance and other biologically based methods for control. Biologically based control methods, such as microbial biological control agents and organic amendments, suffer from inconsistent performance (Bonanomi et al., 2007; Roberts and Kobayashi, 2011) and in the case of organic matter amendments, can take several years before they provide benefit (Termorshuizen et al., 2006; Bonanomi et al., 2007).

Our long-term goal is to develop alternative control measures for damping-off of cucumber caused by *Pythium ultimum* and *Rhizoctonia solani*; control measures applicable for use in both conventional and organic production systems. Breeding programs for resistance to *Pythium* are in their infancy (Louws et al., 2010) forcing growers to rely on pesticides such as metalaxyl. This is problematic as resistance to metalaxyl in *P. ultimum* and other important related soilborne plant pathogens has been reported (Brantner and Windels, 1998; Lamour and Hausbeck, 2001; Parra and Ristaino, 2001; Gracia-Garza et al., 2003). Currently available chemical controls for *R. solani* are not completely effective (Csinos and Stephenson, 1999; Campion et al., 2003; Huang et al., 2012). Although a number of microbial biological control agents have been reported to be effective against soilborne pathogens such as *P. ultimum* and *R. solani*, and some commercial products are available (Martin and Loper, 1999; Fravel, 2005), the development of additional biologically based methods that can be applied in combination with existing control measures may improve disease suppression consistency and efficacy (Lemanceau and Alabouvette, 1991; Pierson and Weller, 1994; Raupach and Kloepper, 1998; Chellemi, 2002).

In prior work we demonstrated the ability to provide significant suppression of *P. ultimum* damping-off on cucumber and other cucurbits with ethanol extracts of *Serratia marcescens* strain N4-5 relative to the nontreated control. We also presented evidence that this suppression was due, at least in part, to prodigiosin (Roberts et al., 2007). Here we test ethanol extracts of additional microbial biological control agents previously shown to produce ethanol-soluble antibiotics active against *P. ultimum* and/or *R. solani* for suppression of damping-off caused by these pathogens. These applications of ethanol are OMRI (Organic Materials Review Institute)-approved for use in organic crop production (www.omri.org/omri-lists). We also test the commercial feasibility of ethanol extracts of strain N4-5 by 1) comparing suppression of *P. ultimum* on cucumber with a commercial seed treatment pesticide and a commercial biological control agent for this pathogen, and 2) determining the shelf life of these ethanol extracts.

2. Materials and methods

2.1. Microbial isolates

Bacterial, fungal, and oomycete isolates used in this study were from the USDA-ARS Sustainable Agricultural Systems Laboratory culture collection and are listed in Table 1.

2.2. Preparation of microbial extracts

Ethanol cell extracts were prepared from *S. marcescens* N2-4, N4-5, ATCC 274, and ATCC 8100 grown on Peptone Glycerol (PG) agar plates at 28 °C as described previously (Matsuyama et al., 1985; Roberts et al., 2007). Strains grown on PG agar plates for 3 days at 28 °C were also extracted with ethyl acetate. Briefly, cell mass from PG agar plates was suspended in ethyl acetate (10 mL per plate), and passed through a 0.2 μ filter. All extracts were dried under nitrogen and the residue resuspended as indicated prior to use. Extracts were made from *Burkholderia cepacia* BC-1, *B. cepacia* BC-2, and *Burkholderia ambifaria* BC-F grown in 600 mL Nutrient broth for 48 h at 28 °C and 250 rpm. Cultures were centrifuged at 8700 × g for 10 min and the supernatant discarded. The cell pellet was suspended in 100 mL ethanol or ethyl acetate and sonicated (Vibra Cell, Sonics and Materials, Danbury, CT) for 3 min, centrifuged again, and the cell pellet sonicated in the same solvent again.