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Antifungal effects of compost tea microorganisms on tomato pathogens

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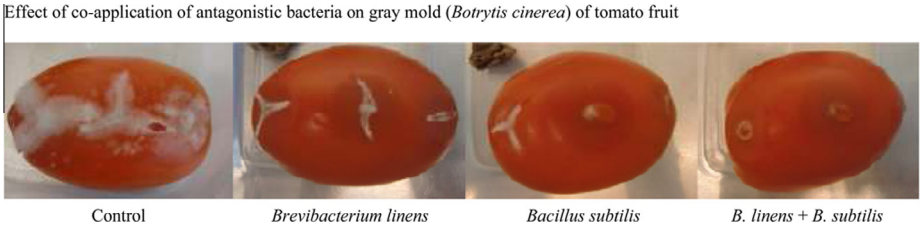
HIGHLIGHTS

- Bacteria from compost tea reduced mycelial growth of tomato pathogens.
- *Bacillus subtilis* and *Brevibacterium linens* inhibited disease on tomato fruit.
- Combined bacterial application revealed synergistic effects.
- *Bacillus subtilis* produced antifungals from the surfactin family of lipopeptides.

GRAPHICAL ABSTRACT



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ABSTRACT

Compost teas are fermented aqueous extracts of composted materials that are used for their ability to control plant pathogens. It had been previously reported that this inhibition by compost teas is at least partially attributed to the presence of live microorganisms. In this study, the inhibitory effects of bacteria from suppressive compost tea were examined against mycelial growth of *Alternaria solani* and *Botrytis cinerea* as well as disease development on tomato fruit. Isolation of antifungal extracts and identification of antifungal compounds from the most effective bacterial strains were also performed. Results showed that the bacteria had the ability to greatly inhibit the mycelial growth of *B. cinerea* and/or *A. solani* by up to 70%. The two most effective isolates, *Brevibacterium linens* (IC 10) and *Bacillus subtilis*, showed that co-application of bacterial antagonists (5×10^5 or 5×10^6 cells) with the pathogens on tomato fruit demonstrated inhibition of the development of *B. cinerea* lesions by up to 61%. A preventive application of the bacteria (5×10^5 or 5×10^6 cells) was more effective than co-application, allowing a significant reduction in lesions of *A. solani* and improving efficacy of low bacterial concentrations in reducing *B. cinerea* lesions. A combined *B. linens*/*B. subtilis* treatment was generally more inhibitory than either bacterium alone indicating possible synergistic effects. Antifungal compounds, including surfactins, were found in the bacterial extracts indicating that antibiosis is a main mechanism of action.

1. Introduction

Synthetic fungicides remain the most widely used control measure against fungal plant diseases. Although relatively effective, synthetic fungicides have two major drawbacks: their generally widespread lack of long-term efficacy caused by the development of resistance in plant pathogens (Avis, 2007) and their potential adverse effects on human health and the environment (Kolaei et al., 2013). In addition to these drawbacks in field or greenhouse cropping systems, pesticide treatments in warehouses and cold storage units have the added constraints of few newly registered synthetic chemicals as well as few replacements for some banned chemicals (Janisiewicz and Korsten, 2002). Therefore, there remains an urgent need for efficient and reliable pre- and post-harvest plant disease control measures.

A possible alternative to synthetic chemical fungicides is to exploit the antimicrobial activities of compost teas. Compost teas, which are considered safer for health and the environment

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(Siddiqui et al., 2009), are fermented watery extracts of composted materials that can be used for the control of plant pathogens (Litterick et al., 2004). Recently, compost teas from sheep manure compost showed antimicrobial activities against phyllosphere (Koné et al., 2010) and rhizosphere (Dionne et al., 2012) pathogens of tomato (*Solanum lycopersicum* L.) plants. These composts teas did not contain human pathogens (Koné et al., 2010). This and other work indicated that the microbial populations within compost teas were the main factor responsible for the inhibitory effects of compost teas (Scheuerell and Mahaffee, 2004, 2006; Diánez et al., 2006; Gea et al., 2009; Koné et al., 2010; Dionne et al., 2012).

Beneficial microbial antagonists in suppressive compost teas are reported to control plant pathogens through one or more biological control mechanisms. Indeed, the microorganisms present in the tea may act as pathogen antagonists through their ability to compete for nutrients and/or space (Al-Mughrabi et al., 2008), to destroy pathogens by parasitism (El-Masry et al., 2002), to induce systemic resistance in plants (Zhang et al., 1998), and/or to produce antimicrobial compounds (*i.e.*, antibiosis). Microbial antagonists presumably using antibiosis as a mechanism of biocontrol were isolated from sheep manure compost tea (Koné et al., 2010; Dionne et al., 2012) by targeting their ability to produce extracellular antimicrobial compounds.

In this study, the antagonistic bacteria isolated from sheep manure compost tea were tested (i) to assess their inhibitory activity on mycelial growth of fungal pathogens, (ii) to evaluate their ability to suppress disease *in vivo*, and (iii) to isolate and identify antifungal compounds from the most effective bacterial strains. This study was conducted with the plant pathogenic fungi *Alternaria solani* Sorauer and *Botrytis cinerea* Pers.:Fr. *A. solani* causes disease of potato (*Solanum tuberosum* L.), eggplant (*Solanum melongena* L.), pepper (*Capsicum annuum* L.), and tomato (*S. lycopersicum* L.) and is generally referred to as early blight disease (Zhao et al. 2008). *B. cinerea* is the causal agent of gray mold on numerous plant species including tomato (Cantu et al., 2009).

2. Materials and methods

2.1. Microbial material

The antagonistic bacteria were isolated from a compost tea prepared from sheep manure compost (Koné et al., 2010; Dionne et al., 2012). *Advenella incenata* Coenye et al. isolates IB48 (GenBank Accession number KJ689306) and IC6 (KJ689309), *Aminobacter aminovorans* (den Dooren de Jong) Urakami et al. isolate IB2 (KJ689311), *Bacillus subtilis* (Ehrenberg) Cohn isolate IC23 (KJ689307), and *Brevibacterium linens* (Wolff) Breed isolates IB16 (KJ689310) and IC10 (KJ689308) were identified using nucleotide sequence data from the small subunit (16S) of ribosomal RNA. These bacteria were cultured from freeze-dried or glycerol stocks and maintained on tryptic soy agar (TSA, Becton Dickinson, Sparks, MD). The fungal plant pathogens *A. solani* and *B. cinerea* were provided by the Laboratoire de diagnostic en phytoprotection (MAPAQ, Québec, Canada) and were cultured from freeze-dried stocks and maintained on potato dextrose agar (PDA, Becton Dickinson).

2.2. Effect of compost tea antagonists on the mycelial growth of *B. cinerea* and *A. solani*

Bacteria were transferred from TSA Petri dishes to 15-mL conical tubes containing 5 mL of tryptic soy broth (TSB, Becton Dickinson) using an inoculation loop. The bacteria were grown in an incubator-shaker for 48 h at 26 °C and 175 rpm. Following incubation, bacteria were adjusted to 5×10^7 cells/mL with sterile distilled water using a hemacytometer. Ten microliter of each bacterial suspension was inoculated as a single streak (1 cm wide) at the four cardinal points of a Petri dish containing PDA. The distance between two opposite streaks was 6.5 cm (for *B. cinerea* trials) and 4.5 cm (for *A. solani* trials). The dishes were incubated for 48 h at 26 °C in the dark. Following this incubation period, the center of each dish was inoculated with a 0.5-cm (diameter) agar plug containing actively growing mycelia from the thallus margins of either *B. cinerea* or *A. solani*. PDA inoculated with either *B. cinerea* or *A. solani*, but without the bacteria served as controls. The dishes were incubated another 48 h at 26 °C. The mycelial growth of *B. cinerea* and *A. solani* was noted after the incubation period and expressed as the average of two perpendicular diameters of the thallus. The experiments were conducted as complete block designs with five replicates.

2.3. Effect of *B. linens* (IC10) and *B. subtilis* on disease on tomato fruit

2.3.1. Effect of co-application of *B. linens* (IC10) and *B. subtilis* on disease of tomato fruit

B. linens (IC10) and *B. subtilis* were transferred from TSA Petri dishes to 15-mL conical tubes containing 5 mL of tryptic soy broth (TSB, Becton Dickinson) using an inoculation loop. The bacteria were grown in an incubator-shaker for 48 h at 26 °C and 175 rpm. Following incubation, bacteria were recovered by centrifugation (5300×*g* for 20 min). The supernatant was removed and the pellet was washed with 5 mL of sterile distilled water and recentrifuged. The supernatant was discarded and the remaining pellet served as inoculum stocks. The bacterial inocula were adjusted to 5×10^7 cells/mL or 5×10^8 cells/mL with sterile distilled water using a hemacytometer.

A. solani and *B. cinerea* spores were individually collected by flooding mycelia grown for 1 (*A. solani*) and 2 weeks (*B. cinerea*) on PDA with 1 mL sterile distilled water and by gently scraping the surface of the mycelia with a sterile glass rod to dislodge the spores. The spore suspensions were collected by aspiration with a micropipette, placed in a 1.5-mL microcentrifuge tube and diluted to 5×10^4 conidia/mL based on hemacytometer counts of the stock suspension.

Tomato fruit (cultivar 'Roma') were surface sanitized in 70% ethanol for 15 min, rinsed with sterile distilled water, and allowed to air dry. The skin of each tomato was pierced with a sterile needle to provide a wounded inoculation site (diameter 1.5 mm, depth 7 mm). Each wound was inoculated individually with 10 μL of fungal spore suspension (*A. solani* or *B. cinerea*). Following fungal inoculation, wounds were immediately treated with either 10 μL of sterile distilled water (controls), 10 μL of *B. linens* IC10 cell suspension, 10 μL of *B. subtilis* cell suspension or 5 μL each of *B. linens* IC10 and *B. subtilis*. Suspensions were used at the concentration indicated above to provide 1000:1 and 10,000:1 bacterial cell/fungal spore ratios as previously described (Siripornvisal, 2010). In addition, 10 μL of each bacterial cell suspension was tested in the absence of fungal spores to assess the potential effect of the bacteria on the tomato fruit. Each tomato fruit was individually placed in a plastic container with a moistened paper towel to maintain relative humidity (RH > 95%). Containers were sealed and incubated in the dark at 20 °C for five (*B. cinerea*) to seven (*A. solani*) days. Symptoms of disease were measured as the average of two perpendicular diameters of the visible surface lesions following 5 and 7 days of incubation for *A. solani* and 3 and 5 days of incubation for *B. cinerea*. The experiments consisted of randomized complete block designs with three wounds per tomato and three replicates per treatment. The experimental unit was one tomato fruit in an individual container. The experiments were repeated twice.