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Fusarium head blight biological control with *Lysobacter enzymogenes* strain C3

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Abstract

Fusarium head blight (FHB), caused by *Fusarium graminearum* (= *Gibberella zeae*), is a destructive disease of wheat for which biological controls are needed. *Lysobacter enzymogenes* strain C3, a bacterial antagonist of fungal pathogens via lytic enzymes and induced resistance, was evaluated in this study for control of FHB. In greenhouse experiments, chitin broth cultures of C3 reduced FHB severity to <10% infected spikelets as compared to >80% severity in the controls in some experiments. C3 broth cultures heated to inactivate cells and lytic enzymes, but retaining the elicitor factor for induced resistance, also were effective in reducing FHB severity, suggesting induced resistance is one mechanism of action. C3 broth cultures also were effective when applied in highly diluted form and when applied 1 week prior to pathogen inoculation. When applied to 8 cultivars of hard red spring wheat in the greenhouse, C3 treatments reduced FHB in 5 cultivars but not in the others. These findings also are consistent with induced resistance. Protection offered by C3 treatments, however, was not systemic and required that C3 be applied uniformly to all susceptible florets. Field tests were conducted in South Dakota and Nebraska to evaluate the efficacy of C3 chitin broth cultures in spring and winter wheat, respectively. In experiments involving two hard red spring wheat cultivars, treatment with C3 reduced FHB severity in 'Russ' but not in 'Ingot'. In three other field experiments comparing C3, the fungicide tebuconazole, and the combination of C3 and tebuconazole, treatments with the bacterial culture alone and the fungicide alone were inconsistent across experiments, each treatment being ineffective in controlling FHB in one experiment. The biocontrol agent–fungicide combination was more consistently effective, reducing FHB incidence or severity in all three experiments. Thus, the potential for using *L. enzymogenes* C3 as a biological control agent for FHB was demonstrated along with a number of factors that might affect control efficacy in the field.

Keywords: *Fusarium graminearum*; *Gibberella zeae*; Fusarium head blight; Scab of wheat; *Lysobacter enzymogenes*; Biological control; Induced resistance; Biocontrol agent–host interactions; Bacteria

1. Introduction

Fusarium head blight (FHB) or scab, caused by *Fusarium graminearum* (Schwabe) (teleomorph = *Gibberella zeae* (Schwein.)), is one of the most important diseases of wheat and barley throughout the world. Losses result from depressed grain yield, reduced grain quality, and contamination of harvested grain with the mycotoxin deoxynivalenol, or vomitoxin (McMullen et al., 1997). The use of cultivars with moderate levels of tolerance is an important strategy for the control of FHB in wheat, but is unavailable in some market types and provides only partial control (Bai and Shaner, 1996; Van Ginkel et al., 1996). Fungicides are needed to control FHB under high inoculum pressure and favorable environmental conditions, or when resistance is unavailable, but they have not been consistently effective in controlling FHB and in reducing deoxynivalenol formation (McMullen et al., 1997).

The need for alternative disease management strategies to augment host resistance and fungicides has driven a worldwide search for biological control agents to protect

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flowering heads against FHB (da Luz et al., 2003). Strains of *Bacillus* spp. and *Pseudomonas* spp. have been the most commonly investigated bacterial agents (da Luz et al., 2003; Khan et al., 2004; Schisler et al., 2002.) Yeast strains, including those of *Cryptococcus* spp., also have proven to be promising biocontrol agents against FHB (Khan et al., 2001). Efficacy in reducing FHB severity or deoxynivalenol accumulation with bacterial and yeast strains has been documented, but consistent field performance over time and across locations is difficult to achieve (da Luz et al., 2003; Khan et al., 2004; Schisler et al., 2002). Nevertheless, biological control appears to be a useful tool when integrated with fungicide applications and host resistance (da Luz et al., 2003; Khan et al., 2004).

In this study, we investigated, as a potential biocontrol agent for FHB, *Lysobacter enzymogenes* strain C3, which was isolated from grass foliage and originally reported to be a strain of *Stenotrophomonas maltophilia* (Giesler and Yuen, 1998), but repositioned subsequently into the closely related species *L. enzymogenes* (Sullivan et al., 2003). This bacterial agent is taxonomically and mechanistically unique from all other biocontrol agents of FHB. *Lysobacter* spp. are Gram-negative bacteria with gliding motility (Christensen and Cook, 1978). C3 suppressed a number of plant diseases incited by fungi in field experiments (Giesler and Yuen, 1998; Yuen and Zhang, 2001; Yuen et al., 2001; Zhang and Yuen, 1999). While the mechanisms involved in control of FHB by bacterial and yeast strains have not been clearly established, most are presumed to inhibit the disease through the production of antibiotics or through competition for nutrients (da Luz et al., 2003). In contrast, C3 has the potential to inhibit fungi through lytic activity from chitinases (Zhang and Yuen, 2000b; Zhang et al., 2001) and β-1,3-glucanases (Palumbo et al., 2005), in addition to antibiotics (Zhang and Yuen, 2000a; Li et al., 2006). Induced resistance via a heat-stable elicitor also can play a role in biocontrol by C3, as demonstrated against Bipolaris leaf spot in turfgrass and wheat (Kilic-Ekici and Yuen, 2003). In that study, systemic resistance was induced when C3 was applied to roots, but only localized protection occurred when the bacterium was applied to foliage. The highest levels of disease control using C3 were achieved with whole chitin broth cultures, containing cells and culture fluid, as compared to applying cellular or fluid fractions alone (Yuen and Zhang, 2001; Yuen et al., 2001; Zhang and Yuen, 2000a); this can be attributed partly to the fluid fraction containing excreted antifungal compounds (Zhang and Yuen, 2000b) and factors responsible for eliciting resistance (Yuen, unpublished data).

Given the biological control attributes of C3, the bacterium presented unique opportunities as a candidate biological control agent against FHB. The potential to induce resistance, in particular, could be an advantage in controlling FHB because induced resistance, if expressed systemically, is relatively independent of the population and spatial constraints that limit antagonism (Van Loon et al., 1998). On the other hand, studies on other systems of induced resistance found different levels of response among genotypes of a plant species to an inducing agent (Leeman et al., 1995; Van Loon et al., 1998), and this factor could potentially reduce the usefulness of induced resistance. The objectives of this study were: (i) to determine if C3 could be efficacious on wheat for controlling FHB, (ii) to assess the degrees to which biological control of FHB by C3 could be affected by spatial distribution of the bacterium on wheat plants and by wheat genotype, and (iii) to determine if the combination of C3 with a fungicide would provide consistent control of FHB.

2. Materials and methods

2.1. Microbiological methods

Strain C3 was routinely cultured on tenth-strength tryptic soy agar (10% TSA) from a stock culture stored at −80 °C. To produce inoculum of C3 for treating plants in greenhouse and field experiments, the strain was grown in chitin broth (Zhang and Yuen, 2000b) for 7 days at 25 °C, with shaking at 180 rpm, producing ca. 10^9 CFU/ml. Prior to use, the cultures were filtered through four layers of cheesecloth to remove large particulate matter. As a rifampicin-resistant strain of C3 was used in all experiments, post-application populations of C3 on treated plant parts were determined by plating dilutions of plant washes onto 10% TSA amended with rifampicin and cycloheximide, each at 100 mg/L. Colonies were counted after 2 days incubation at 25 °C and population levels expressed as $\log_{10}$ CFU.

To produce pathogen inoculum for greenhouse experiments, *F. graminearum* NE1, an isolate from wheat, was cultured on half-strength potato dextrose agar for 10 days at 25 °C, and macroconidia then were collected from the plates in sterile distilled water. The conidial suspensions were filtered through Miracloth (Calbiochem, La Jolla, CA) to remove mycelial fragments and diluted to 5×10^4 conidia/ml, using a hemacytometer to measure spore density.

2.2. General greenhouse experimental methods

Unless stated otherwise, these methods were used to assess biocontrol efficacy in greenhouse experiments. Hard red spring wheat 'Bobwhite' (FHB susceptible) was grown in 15-cm-diameter pots (four plants per pot) containing a steam-pasteurized potting medium (Sharpsburg silty clay loam, vermiculite, and sand mixed in equal volumes). The plants were maintained in a greenhouse with daily watering and twice weekly fertilization with a nutrient solution (20-10-20 Peat-Lite Special, Scotts-Sierra Horticultural Co., Marysville, OH). At anthesis (Feekes stage 10.51), when yellow anthers exserted from florets, treatments were sprayed onto heads to run-off (approximately 5 ml/head) using a spray atomizer connected to an air source. The same treatment was applied to all heads (usually six) in a