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Biological control of *Fusarium graminearum sensu stricto*, causal agent of Fusarium head blight of wheat, using formulated antagonists under field conditions in Argentina

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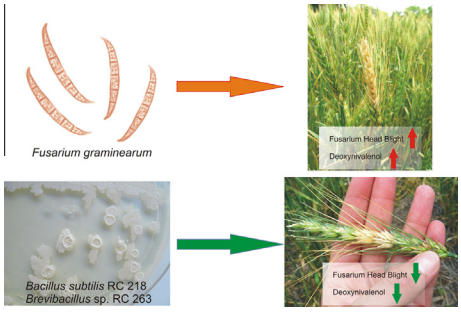
HIGHLIGHTS

- Biological control of Fusarium head blight in wheat.
- Two bacterial strains as biocontrol agents against *Fusarium graminearum*.
- Biocontrol agents reduced disease severity by 42–76%.
- Biocontrol agents reduced deoxynivalenol on spikes to undetectable levels.

GRAPHICAL ABSTRACT



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ABSTRACT

Fusarium head blight (FHB) mainly caused by *Fusarium graminearum* is a devastating disease that causes extensive yield and quality losses to wheat in humid and semi-humid regions of the world. The biocontrol effect of two bacterial strains on FHB incidence, severity and deoxynivalenol (DON) accumulation in wheat was evaluated in field trials during 2010 and 2011 at Marcos Juarez, Córdoba province, Argentina. *Bacillus subtilis* RC 218 and *Brevibacillus* sp. RC 263 applied at anthesis period were evaluated through several combinations of cell type, strains, inoculum density (10^4 and 10^6 cfu/ml) and physiological modification. A significant and consistent biocontrol effect on FHB severity and DON contamination was observed in all the evaluated treatments during both 2010 and 2011 field trials. Reduction in FHB severity ranged 62–76% and 42–58% for 2010 and 2011 field trials, respectively. When evaluating the effect of the combined strains ($10^4 + 10^4$ and $10^6 + 10^6$ cfu/ml), a better biocontrol effect was observed in 2010 field trial. After biocontrol treatments, no DON accumulation was observed in wheat heads; meanwhile in control plots an average of 1372 μg/kg DON was detected during the two trials. FHB incidence was significantly reduced by biocontrol treatments during the 2010 field trial but not during the 2011 field trial. The results showed the effectiveness of the two formulated biological control agents in reducing both FHB severity and DON accumulation by *F. graminearum* under semi controlled field conditions.

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1. Introduction

Fusarium head blight (FHB) mainly caused by *Fusarium graminearum sensu stricto* is a devastating disease that causes extensive yield and quality losses to wheat in humid and semi-humid regions of the world. Besides the economic losses due to reduction in grain yield, the main problem is the potential mycotoxin contamination of wheat mainly with deoxynivalenol (DON) (McMullen et al., 2012). During the last 50 years, several epidemics of FHB of varying degrees of severity have occurred in Argentina and *F. graminearum* "*sensu stricto*" was isolated as the main pathogen associated with FHB (Dalcero et al., 1997). In 1993, during a severe FHB outbreak, the highest estimated losses reached 50% in areas with no-tillage after maize crops. The extent of the damage was magnified by a considerable loss in grain trading value resulting from low grain weight, the presence of scabby grains, and DON contamination (Kikot et al., 2011; Ramirez et al., 2007).

Different strategies are used to reduce the impact of FHB including crop rotation, tillage practices, fungicide application and planting less susceptible cultivars. Among these strategies, fungicide control seems to be the most effective (Homdork et al., 2000; Mesterházy et al., 2011), although it was observed that certain fungicides could increase DON content on grains (Ramirez et al., 2004) and pathogens can generate fungicide resistance (Yuan and Zhou, 2005). Genetic resistance is also a viable option, but at present no successful results have been achieved (Bai et al., 2000, 2001; Buerstmayr et al., 2009; Miedaner and Korzun, 2012; Talas et al., 2012). None of these strategies by themselves are able to reduce the impact of FHB (Dill-Macky and Jones, 2000; Hollins et al. 2003). Biological control offers an additional strategy and can be used as part of an integrated management of FHB.

Anthesis is the stage of greatest susceptibility for *F. graminearum* infection. It is presumed that anthers are the common pathogen entry route into the plant (McMullen et al., 2012). Thus, antagonists with high ecological competence in this niche may prevent infection during anthesis when conditions for the pathogen and antagonists, temperature and humidity, are adequate (Khan et al, 2001). In fact, biocontrol agents (BCA) against the pathogen causing FHB have been evaluated using this application strategy. Nevertheless, formulation of a BCA applied during anthesis has not been fully developed since applications were done with bacterial broths and culture supernatants (da Luz et al., 2003; Khan et al., 2004; Khan and Doohan, 2009; Palazzini et al., 2007; Schisler et al., 2006).

Spray-drying technology for BCAs after their mass production in liquid fermentation systems allows a high processing rate with almost continuous production at low operation costs and short operation time so that production costs are 30–50 fold lower compared to freeze drying technologies (Lian et al., 2002; Silva et al., 2005; Xueyong et al., 2008). However, the production process can drastically affect the viability of biocontrol agents, especially bacteria and yeasts (Abadias et al., 2005; Silva et al., 2005). In order to conserve viability during the drying process, several attempts of physiological improvement have been done to increase desiccation tolerance during this process (Cañamas et al., 2007; Montazeri and Greaves, 2002; Teixidó et al., 2006). In some studies a better FHB control was achieved after improving the BCA quality by changing carbon and nitrogen ratios during fermentation (Zhang et al., 2005), including additives such as chitosan (Khan and Doohan, 2009) and enhancing bioformulated survival by nutrient amendments (Schisler et al., 2004). In previous studies, we have demonstrated that physiologically modified strains of *Bacillus subtilis* RC 218 and *Brevibacillus* sp. RC 263 were effective biocontrol agents to control FHB under greenhouse conditions (Palazzini et al., 2009).

The objectives of the present study were to evaluate the effect of two formulated antagonists (*B. subtilis* RC 218 and *Brevibacillus* sp. RC 263) applied alone or in combination, at two level doses (10^4 and 10^6 cfu ml^{-1}) on: - Fusarium head blight incidence and severity and DON accumulation on wheat spikes under field conditions.

2. Materials and methods

2.1. Biocontrol strains, biomass production and formulation

B. subtilis RC 218 and *Brevibacillus* sp. RC 263 strains used in this study were originally isolated from wheat anthers as potential biocontrol agents against *F. graminearum* in Argentina (Palazzini et al., 2007, 2009). These strains are maintained in the culture collection Department of Microbiology and Immunology at Universidad Nacional de Río Cuarto; Río Cuarto, Córdoba, Argentina). Biomass of *B. subtilis* RC 218 and *Brevibacillus* sp. RC 263 was produced in liquid basic medium (sucrose 10 g/l, yeast extract 5 g/l) described by Costa et al. (2001) with an incubation of 48 h at 28 °C in a rotatory shaker (150 rpm). Additionally, liquid media was modified with NaCl (a_W 0.97) for *B. subtilis* RC 218 biomass production in order to obtain a physiological improvement of the strain by intracellular accumulation of betaine (Palazzini et al., 2009). After biomass production, cells were centrifuged at 10,000 rpm for 5 min, washed with sterile distilled water, centrifuged again and, finally, resuspended in sterile skimmed milk (20% w/v), as a protective agent during spray drying as the final formulation step (Palazzini et al., 2010).

Biomass of *B. subtilis* RC 218 was also produced in a 50 l fermentor by Bio-ferm GmbH (Tulln, Austria) in order to obtain bacterial spores. These bacterial spores were freeze dried and also tested in the field trials.

2.2. Pathogen inoculum production

Two strains of *F. graminearum*, RC276 and KRC7, were used in the field trials. These strains were isolated from head blight infected ears from commercial fields located in Pergamino, Buenos Aires, Argentina. Toxigenic profiles were determined in a previous study (Palazzini et al., 2007). *F. graminearum* conidia were produced in Mung bean broth (Rosewich Gale et al., 2002). After 7–10 days of incubation at 25 °C and 200 rpm on a rotatory shaker, cultures were centrifuged (7000 rpm; 5 min), resuspended in sterile distilled water plus Tween 80 (0.05%) and filtered through sterile gauze to obtain a conidia suspension. Macroconidia concentration was determined using a haemocytometer and conidia concentration was adjusted to 5×10^5 conidia/ml (1:1 mixture of RC276 and KRC7 strains).

2.3. Field trials

Two field trials were conducted in Marcos Juarez, Córdoba province, Argentina, during the 2010 and 2011 harvest seasons. The brad wheat (*Triticum aestivum* L.) cultivar BioInta 1005 (susceptible to *F. graminearum*) was sown at the end of July during both trials. During the 2010 harvest season, the experimental plots consisted of two rows (1 m/row, 0.2 m between rows; 80 heads per plot) with three replicates per treatment. During 2011 field trial, the experimental plots consisted of 3 rows (2 m/row, 0.2 m between rows; 250 heads per plot) with three replicates per treatment. The experiments were done in a random block design with 1 m separation between plots. Temperature in the field plots was monitored by an Agro-climatic station located in the experimental fields.