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Bacterium-mediated control of Fusarium head blight disease of wheat and barley and associated mycotoxin contamination of grain

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ABSTRACT

Fusarium culmorum causes Fusarium head blight (FHB) disease of cereals, resulting in yield loss and contamination of grain with the trichothecene mycotoxin, deoxynivalenol (DON). In a test for potential disease control organisms, *Pseudomonas fluorescens* strains MKB 158 and MKB 249 and *Pseudomonas frederiksbergensis* strain 202 significantly reduced both the severity of FHB disease symptoms caused by *F. culmorum* on wheat and barley ($\geqslant$23%; $P \leqslant 0.050$) and the disease-associated loss in 1000-grain weight ($\geqslant$16%; $P \leqslant 0.050$) under both glasshouse and field conditions when applied 24 h pre-pathogen inoculation. Glasshouse studies showed that these bacteria were more effective in controlling disease when applied 24 h pre- as opposed to 24 h post-pathogen inoculation. The most striking finding was that, in the *F. culmorum*-inoculated field trials, treatment with either of the two *P. fluorescens* strains (MKB 158 or MKB 249) also significantly reduced the DON levels in wheat and barley grain (74–78%; $P \leqslant 0.050$). This is the first report detailing the ability of fluorescent pseudomonad bacteria to control FHB disease and simultaneously reduce mycotoxin contamination of wheat and barley under field conditions.

1. Introduction

Fusarium species, including *Fusarium graminearum* Schwabe {teleomorph: *Gibberella zeae* (Schw.) Petch} and *F. culmorum* (W.G. Smith) Sacc., cause root rot, seedling blight, foot rot and head blight (Fusarium head blight or FHB) diseases of small-grain cereals (Miedaner, 1997). These diseases are economically important because they cause a reduction in yield and grain quality. FHB disease caused by these pathogens also results in the contamination of grain with mycotoxins, mainly deoxynivalenol (DON) and zearalenone (Rocha et al., 2005). Resistant cultivars and appropriate crop management practices are the most commonly used strategies for the control of FHB disease (Bai and Shaner, 1994). The fungicides fludioxonil, benomyl, tebuconazole, azoxystrobin and mancozeb were reported to be effective against FHB disease of both wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) under field conditions (Jones, 2000; Ioos et al., 2005; Ellner, 2006). There is some evidence that the fungicides tebuconazole and azoxystrobin could enhance mycotoxin production by *Fusarium* species (Gareis and Ceynova, 1994; Simpson et al., 2001; Ramirez et al., 2004).

Several researchers have investigated the potential of biological control agents to control FHB disease. The bacteria *Bacillus subtilis* {(Ehrenberg 1835) Cohn 1872} strain AS 43.3, *Pseudomonas* sp. strain AS 64.4 and the yeasts *Cryptococcus* sp. strains OH 71.4 and OH 181.1 and *C. nodaensis* (Sato et al., 1999) strain OH 182.9 gave $\geqslant$25% control of FHB disease of wheat under both glasshouse and field conditions (Schisler et al., 2002, 2006). However, they did not significantly reduce the DON content of grain. In another field-based study, Jochum et al. (2006) reported that the bacterium *Lysobacter enzymogenes* (Christensen and Cook 1978) strain C3 could reduce FHB disease severity in wheat, but its performance was cultivar (cv.)-dependent. Nourozian et al. (2006) found that, under glasshouse conditions, treatment of heads of FHB-susceptible wheat cv. Falat with a *Streptomyces* sp. (strain 3: not identified to species level) reduced both FHB disease severity and associated loss in grain weight by approximately 50%. Palazzini et al. (2007) have reported two promising biocontrol agents (*Brevibacillus* sp. strain BRC 263 and *Streptomyces* sp. strain BRC 87B) which reduced FHB disease severity by up to 70% and prevented DON contamination of grain, under glasshouse conditions. We previously showed that isolates of four fluorescent *Pseudomonas* species {*P. fluorescens* (Migula 1895 AL) strains MKB 100, MKB 158 and MKB 249 and *P. frederiksbergensis* (Andersen et al., 2000) strain 202} reduced the development of seedling blight disease of wheat cv. GK-Othalom and barley cv. Lux caused by *F. culmorum* by $\geqslant$53% under glasshouse conditions (Khan et al., 2006). In that study, however, we did not evaluate the efficacy of those or other bacteria against FHB disease.

In this study, we evaluated the potential of seven bacteria to control FHB disease of wheat and barley under glasshouse conditions. Culture filtrate of one such bacterium (*P. fluorescens* strain

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MKB 158) was previously shown to reduce the development of Fusarium seedling blight symptoms on wheat and barley (Khan et al., 2006); therefore we also tested the effect of such culture filtrate on FHB disease development under glasshouse conditions. Based on the glasshouse results, *P. fluorescens* strains MKB 158 and MKB 249 and *P. frederiksbergensis* strain MKB 202 were chosen and tested under field conditions. The effect of these three bacteria on the accumulation of the mycotoxin DON in wheat and barley grain was evaluated under field conditions and the effect of *P. fluorescens* strain MKB 158 on DON accumulation in wheat and barley grain was also evaluated under glasshouse conditions.

2. Materials and methods

2.1. Plant, fungus and potential biocontrol agents

The hard red winter wheat (*Triticum aestivum* L. cv. GK-Othalom) (kindly supplied by Lazlo Hornok, Agricultural Biotechnology Center, Godollo, Hungary) and the two-row spring barley (*Hordeum vulgare* L. cv. Lux) (kindly supplied by Powerseeds, Kildare, Ireland) were used in this study. The fungal isolate used in this study was *Fusarium culmorum* (W.G. Smith) Sacc. strain FCF 200 (kindly supplied by Dr. Paul Nicholson, John Innes Center, Norwich, UK). The bacteria used in this study, an unidentified isolate MKB 25, *Pseudomonas fluorescens* strains MKB 100, MKB 158 and MKB 249, *Acinetobacter* sp. strain MKB 121, *P. frederiksbergensis* strain MKB 202 and *Chryseobacterium* sp. strain MKB 277 were chosen based on their consistent ability to improve coleoptile growth of *F. culmorum*-inoculated seed in a previous Fusarium seedling blight screening experiment (Khan et al., 2006). Also, *P. fluorescens* strains MKB 100, MKB 158 and MKB 249, *P. frederiksbergensis* strain MKB 202 and *Chryseobacterium* sp. strain MKB 277 all significantly reduced Fusarium seedling blight disease symptoms on both wheat (cv. GK-Othalom) and barley (cv. Lux) by at least 23%, relative to LB-treated control plants inoculated with *F. culmorum*. The culture and maintenance of *F. culmorum* strain FCF 200, the bacteria, and the preparation of bacterial culture filtrate were as described earlier (Khan et al., 2006). Briefly, *F. culmorum* conidia were harvested from 7-day-old Mung bean broth cultures (100 ml cultures incubated at 25 °C, 150 rpm) and adjusted to a final concentration of 5×10^4 spores (conidia) ml^{-1} with 0.2% Tween 20. *P. fluorescens* cells were produced in Luria-Bertani (LB) broth (Luria and Burrows, 1957) (100 ml cultures, incubated for 24 h at 25 °C, 180 rpm) and the cell concentration was adjusted to 7 log10 CFU ml^{-1} with LB broth. Filtrate was obtained using 0.2 μm nitro-cellulose filters (Millipore Corp., Billerica, MA).

2.2. Glasshouse evaluation of the potential biocontrol agents

Glasshouse trials of the bacteria for FHB control were conducted using the winter wheat cv. GK-Othalom and spring barley cv. Lux during autumn 2003 and 2004. Plants were grown in a non-climate-controlled glasshouse. Two grams of NPK (10-10-20) fertilizer (Agrifert, Switzerland) was added to each pot at growth stages (GS; Zadoks et al., 1974) 20 (start of tillering) and 30 (start of stem elongation). To control aphids and powdery mildew, the aphicide Decis Quick® (25 g deltramethrin l^{-1}, 0.25 l Ha^{-1}) and the fungicide Corbell (750 g fenpropimorph l^{-1}, 1 l Ha^{-1}) were sprayed at GS 25 (mid tillering) and GS 28 (late tillering), respectively. Plants were irrigated every two days with water (applied directly to soil). The glasshouse temperature between the time of treatment (mid-anthesis) and harvest was 24–28 °C (in both 2003 and 2004). Artificial lighting was not used in these experiments; noon light intensity between the time of treatment and harvest ranged from 140–930 μmol m^{-2} s^{-1}.

In each trial, for both wheat and barley, each treatment combination was applied to 10 heads (1 head per plant) at mid-anthesis. Heads subjected to treatments were secondary heads and were selected on the basis that they were at the same growth stage, i.e. GS 65 (mid-anthesis) (Zadoks et al., 1974). Heads were sprayed to run-off (approximately 4 ml) with bacterial cultures, (7 log10 CFU ml^{-1}), or culture filtrate of *P. fluorescens* strain MKB 158 24 h before or after spray-inoculation to runoff (approximately 4 ml) with conidia of *F. culmorum* strain FCF 200 (at a concentration of 5×10^4 conidia ml^{-1} in 0.2% Tween 20) or with 0.2% Tween 20. All treatments were applied with a 1-l hand-held sprayer (Hozelock, UK). Heads were covered with polythene bags for 48 h following inoculation. Control plants were treated with LB + Tween 20 and LB + *F. culmorum* as negative and positive controls, respectively). As an additional control, plants were also treated with the bacteria alone to assess whether these agents might have a detrimental effect on plant health. Following treatment, plants were arranged in a randomized block design to minimize variability which might arise between treatments due to the location of the plants within the glasshouse environment.

The percentages of bleached spikelets per head were scored at GS 70 (start of milk development), 80 (start of dough development) and 90 (start of ripening) and the Area Under the Disease Progress Curve (AUDPC) was calculated, as described by Shaner and Finney (1977). The effects of the biocontrol agents on AUDPC was calculated as the percent reduction in AUDPC in biocontrol-treated, relative to AUDPC values obtained for positive control plants. When ripe (GS 99), cereal heads were harvested, freeze-dried for 48 h and the number and weight of grains in each head was used to calculate the 1000-grain weight. 1000-grain weight results were used to calculate the percent loss in 1000-grain weight associated with treatment combinations, relative to the 1000-grain weight obtained for negative control samples.

2.3. Small-scale field evaluation of the potential biocontrol agents

Small-scale FHB field trials were conducted in Thornfield, UCD (Dublin, Ireland) in 2004/2005 and 2005/2006 using the wheat cv. GK-Othalom and the barley cv. Lux. Trial plots were 1 m^2 and were separated by a 1 m guard row of oats. Plots were prepared for the trials by applying herbicides Duplosan {CMPP, 600 g l^{-1}, Mecoprop-p 4.3%, 1.5 l Ha^{-1}) (BASF, Ireland) and Oxytril (200 g bromoxynil and 200 g bioxynil l^{-1}, 1.5 l Ha^{-1}) (Bayer Crop Science, UK)}, after which the whole plot was rotavated and all stubble was removed. A 10-10-20 NPK fertilizer (Agrifert, Switzerland) was applied at 4 g per 1 m^2 plot. After a week, eight grams of wheat or barley seed were sown into the 1 m^2 plots. The plots were enclosed in netting to prevent seed loss due to birds. To control aphids and powdery mildew, the aphicide Decis Quick® (25 g deltramethrin l^{-1}, 0.25 l Ha^{-1}) and the fungicide Corbell (750 g fenpropimorph l^{-1}, 1 l Ha^{-1}) was sprayed at GS 25 (mid tillering) and GS 28 (late tillering), respectively. Barley plants reached mid-anthesis on June the 5th in 2004/2005 and June the 20th in 2005/2006. Between mid-anthesis and harvest the average daily temperature was 18.67 °C and 20.09 °C, respectively, and the precipitation was 1.38 and 1.09 mm, respectively (plants received no additional irrigation). Wheat plants reached mid-anthesis on May the 25th in 2004/2005 and June the 7th in 2005/2006. Between mid-anthesis and harvest the average daily temperature was 17.63 °C and 19.56 °C, respectively and the precipitation was 1.32 and 1.37 mm, respectively (plants received no additional irrigation). We didn't use any artificial light for these experiments, but noon light intensity ranged from 276 to 1410 μmol m^{-2} s^{-1} from inoculation (mid-anthesis) to harvest.

Each treatment was applied to 3 plots and 10 heads per plot per treatment at mid-anthesis. Secondary heads at the same growth