Available online at www.sciencedirect.com
ScienceDirect
Biological Control 42 (2007) 281–287
Biological Control
www.elsevier.com/locate/ybcon

Effects of biocontrol agents on *Fusarium verticillioides* count and fumonisin content in the maize agroecosystem: Impact on rhizospheric bacterial and fungal groups

Paola Pereira [1], Andrea Nesci [2], Miriam Etcheverry [*,2]

Orientación Ecología Microbiana, Departamento de Microbiología e Inmunología, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Ruta Nacional 36 Km 601 (5800) Río Cuarto, Córdoba, Argentina

Received 25 October 2006; accepted 23 May 2007
Available online 8 June 2007

Abstract

The present study tested the ability of *Bacillus amyloliquefaciens* and *Microbacterium oleovorans* to reduce *Fusarium verticillioides* populations and fumonisin accumulation in the maize agroecosystem. The impact of releasing these biocontrol agents on rhizospheric bacterial and fungal groups was also evaluated through isolation and identification of culturable microorganisms. When applied as seed coatings at a concentration of 10^7 CFU ml^{-1} both agents were effective in reducing *F. verticillioides* counts and fumonisin B_1 and B_2 content from maize grains. Rhizospheric counts of the pathogen were also decreased by use of *B. amyloliquefaciens* at 10^7 CFU ml^{-1}. Richness and diversity indexes calculated for bacteria and fungi inhabiting the rhizosphere of maize remained unchanged following the addition of both biocontrol agents to seeds. Our research is being continued to further characterize the bacterial and fungal isolates with additional field assays.

Keywords: *Bacillus amyloliquefaciens*; *Microbacterium oleovorans*; *Fusarium verticillioides*; Biocontrol agents; Fumonisins; Maize; Richness; Diversity

1. Introduction

Maize (*Zea mays* L.) is one of the three most important grain crops in terms of world yield. Moreover it represents one of the main crops grown in Argentina (Liendo and Martín, 2004). A variety of toxigenic fungi, including *Fusarium*, *Aspergillus* and *Penicillium*, are frequently reported as fungal genera that colonize maize grains (Chulze et al., 1996; Chulze et al., 1999). *Fusarium* is one of the major genera associated with maize and several toxigenic species including *Fusarium verticillioides* (Sacc.) Nirenberg and *Fusarium proliferatum* (Matsushima) Nirenberg, are prolific fumonisin producers (Fandohan et al., 2003). Fumonisins, mainly B_1 and B_2, are a group of mycotoxins that contaminate maize-based foods and feeds throughout the world. These toxins have been related to several diseases such us equine leukoencephalomalacia (ELEM), porcine pulmonary edema (PPE), liver toxicity in many other animals and esophageal and liver cancer in humans (Chu and Li, 1994; Gelderblom et al., 1991; Harrison et al., 1990; Kellerman et al., 1990; Lino et al., 2004; Marasas et al., 1988; Ross et al., 1990; Thiel et al., 1992). Because of the harmful effects associated with the presence of *Fusarium* species in agricultural crops, the control of *F. verticillioides* colonization and fumonisin production in maize has become a priority area in food safety research (Brown et al., 2001).

The field of biocontrol of soil-borne plant pathogens was initiated many years ago and it is being promoted due to its great contribution to the environment conservation (Winding et al., 2004). Field studies must be done to

* Corresponding author. Fax: +54 0358 4676231.
E-mail address: metcheverry@exa.unrc.edu.ar (M. Etcheverry).
[1] Fellow of Fondo para la Investigación Científica y Tecnológica (FONCyT), Argentina.
[2] Members of the Research Career of Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

1049-9644/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.biocontrol.2007.05.015

acquire an integral view of the interrelationships between plants, pathogens and biocontrol agents to help us evaluate the safety of introducing biocontrol agents at field trials.

Since seed treatments with biocontrol agents represent an appropriate method to suppress plant pathogens in the spermosphere and rhizosphere many biocontrol agents have been applied in the form of seed coatings (Batson et al., 2000; Kerry, 2000; Mao et al., 1998; Nacamulli et al., 1997). The activity of *Bacillus amyloliquefaciens* Ba-S13 (GenBank accession no. AY651023) and *Microbacterium oleovorans* DSM 16091T (GenBank accession no. AJ698725) against colonization of maize by *F. verticillioides* and fumonisin production have been studied in vitro and in greenhouse trials (Cavaglieri et al., 2004, 2005a), but studies of their activity have not been conducted at field levels.

This study was conducted to (1) evaluate the effectiveness of biocontrol agents (BCAs) in reducing *F. verticillioides* counts from the maize rhizosphere and from maize cobs at field level, (2) determine the effects of BCAs on total fumonisins content in maize cobs, (3) analyze the impact of introduced agents on culturable bacterial and fungal species richness and diversity, and (4) evaluate the effects of commonly used agrochemicals on the parameters described above.

2. Materials and methods

2.1. Bacterial strains

Bacillus amyloliquefaciens Ba-S13 (GenBank accession no. AY651023) and *M. oleovorans* DSM 16091T (GenBank accession no. AJ698725) were used in this study. Strains were originally isolated from a commercial maize field and identified on the basis of 16S rDNA gene sequence similarity and additionally identified based on their physiological profiling according to Bergey's Manual of Systematic Bacteriology (Holt, 1993).

Strains were stored at −20 °C in glycerol (30%, v/v) and, when required for experimental use, they were transferred to nutrient agar with 5 μg ml^{-1} of rifampicin, 5 μg ml^{-1} of streptomycin and 0.833 ml l^{-1} of Vitavax-FLO (Carboxin + Thiram). Nutrient broths were also prepared and incubated overnight with shaking at 28 °C until late log phase, after which total viable cells were counted by standard plate count method. Suspensions of 10^7 CFU ml^{-1} were used to introduce bacteria as maize seed coating.

2.2. Treatments—soil characteristics

Four treatments were performed in a commercial maize field located in Río Cuarto, Córdoba province, central region of Argentina (30°57′S latitude, 64°50′W longitude, 562 m altitude) during the maize growing season in 2004–2005. For treatment number one (T1-control) maize (*Zea mays* L.) seeds were submerged in 100 ml of sterile water with 0.833 ml l^{-1} of Vitavax-FLO (Carboxin + Thiram) in 250 ml Erlenmeyer flasks, treatment number two (T2) had the same composition of T1 but with the addition of 0.833 ml l^{-1} of a chemical fertilizer mixture consisting of N(15%)–P_2O_5(30%)–K(0%)–S(10%).

In treatments number three (T3) and four (T4) seeds were submerged in 250 ml Erlenmeyer flasks with 100 ml of 10^7 CFU ml^{-1} of *B. amyloliquefaciens* and 10^7 CFU ml^{-1} of *M. oleovorans*, respectively, with the addition of 0.833 ml l^{-1} of Vitavax-FLO (Carboxin + Thiram) in both cases. Treatments were incubated at 28 °C for 2 h on an oscillatory shaker (100 rpm) to allow bacteria, in T3 and T4, to adhere to seeds.

During late October 2004 eighty seeds from each treatment were planted in a furrow of 5 cm depth, with a distance of 25 cm between seeds and of 50 cm between different furrows. The experimental design was a randomized complete block with three replications of each treatment. Individual plots were 10 m long × 3 m wide (seven rows: four treatment rows + three spacer rows between treatment rows) and were separated by 10 spacer furrows sown with non-treated maize seeds.

Field soil was a sandy loam with a 3–4% gradient (Cantero et al., 1999). Average temperature in the region ranged from 8 °C during the colder months to 25 °C in warmer periods.

2.3. Sampling procedures

In mid-March, 2005 during the harvesting season (150 days after germination), ten plants from each treatment replication were randomly chosen and removed together with adherent soil. Roots were individually placed into plastic bags and transported to the laboratory within 12 h after removal. All cobs were also collected by treatment replication and transported to the laboratory. Samples were stored for a week at 4 °C until their processing.

2.4. Isolation and quantification of F. verticillioides on maize grains

Maize grains were removed from all cobs in each treatment replication and milled. Each milled sample was homogenized and reduced through the technique of quartering up to two 1 kg sub-samples. One sub-sample was used for toxin analysis and the other was separated for fungal identification. From this last sub-sample, triplicate 10 g portions were obtained and added to 90 ml of phosphate-buffered saline (PBS, Oxford Ltd., London, UK) and the mixture was shaken for 10 min in an orbital shaker (80 rpm) and diluted to final concentrations of 10^{-2} and 10^{-3}. Aliquots of 0.1 ml from all dilutions were spread-plated in triplicate on Nash–Snyder medium for selective isolation of *Fusarium* species. Plates were incubated 7 days at 28 °C and total count and count per colony type were done. Colonies were purified on carnation leaf agar (CLA) and *F. verticillioides* was identified according to Nelson et al.