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ScienceDirect
Biological Control 44 (2008) 24–31
Biological Control
www.elsevier.com/locate/ybcon

Antifungal activity of *Penicillium striatisporum* Pst10 and its biocontrol effect on Phytophthora root rot of chilli pepper

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Received 21 March 2007; accepted 8 October 2007
Available online 13 October 2007

Abstract

Penicillium striatisporum Pst10 was isolated from the rhizosphere of chilli peppers. In dual culture agar plate assays, this isolate showed very high antagonistic effects on mycelium growth of *Phytophthora* spp., *Cladosporium cucumerium*, and *Sclerotinia sclerotiorum*. In *in vitro* assays, the toxicity of sterilized liquid culture filtrates (SLCF) of Pst10 grown in potato-dextrose broth (PDB) was tested against *Phytophthora capsici* mycelium growth and sporangia/spore formation or germination. The SLCF completely inhibited mycelium growth and even at a 100-fold dilution led to abnormal mycelium. A 20-fold dilution of SLCF inhibited formation and germination of sporangia and spores. Three antifungal substances were separated by thin-layer chromatography (TLC) from organic solvent extracts of liquid culture filtrate of Pst10. Composted pig manure slightly increased the colonization of the chilli rhizosphere by Pst10. In pot tests, the incidence of Phytophthora root rot of chilli was significantly reduced when artificially infested soil was treated with conidia and SLCF of Pst10.

Keywords: *Penicillium striatisporum*; *Phytophthora capsici*; Antagonistic mechanism; Biocontrol

1. Introduction

Phytophthora root rot (PRR), caused by *Phytophthora capsici* Leonian, is a common and destructive disease of greenhouse and field-grown chilli peppers (*Capsicum frutescens* L.) in China and world-wide. The disease causes serious economic losses of chilli peppers in more than 15 Provinces of China (Zuo, 2002). Shandong Province has suffered major PRR outbreaks in 1996, 1997, and 1998 (Lin et al., 2003). Chilli was grown on more than 1.3 million hectares in 2006 in China. Generally, the incidence of PRR in the pepper fields is 20–30% but in many continuous cropping fields it could be 40–60%, which leads to the estimated annual economic loss of ¥675 million or 96 million USD.

There are a few effective chemical control methods for PRR. There have been considerable efforts to find biological control agents for this disease and several potential candidates have been reported including: *Actinomyces* spp. (Zhu et al., 1995; Lee and Hwang, 2002; Hee et al, 2006; Ezziyyani et al., 2007), *Pseudomonas* spp. (Lee et al., 2003a,b,c; Jung and Kim, 2004; Paul and Sarma, 2006), *Bacillus* spp.(Dai and Guan, 1999; Lee and Hwang, 2002; Jung and Kim, 2003; Qiu et al., 2004; Jung and Kim, 2005), *Trichoderma* spp. (Liu and Lu, 2003; Ezziyyani et al., 2007) and even some fungal endophytes (Kim et al., 2007). The most promising have been the strains of *Penicillium* spp. (Anderson et al., 1988; De Cal et al., 1988, 1990; Fang and Tsao, 1989, 1992, 1995; Harrison and Stewart, 1988; Kharbanda and Dahiya, 1990; Melouk and Akem, 1987; Proksa et al., 1992; Nicoletti et al., 2004;

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doi:10.1016/j.biocontrol.2007.10.005

Pascual et al., 2000; Sztejnberg et al., 1988), with *Penicillium oxalicum* Currie&Thom (Windels, 1981; Windels and Kommedahl, 1978, 1982; De Cal et al, 1997a,b, 1999; Larena et al., 2002; Sabuquillo et al., 2006) being the most intensely investigated.

Penicillium striatisporum Stolk was identified by Stolk (1969), but the only subsequent reports on this fungus were a Japanese patent in 1994 (Morino et al., 1994) and a report by Michael et al. (2005). Both studied medical aspects. There are no reports on the biological control potential of this fungus against plant pathogens. In this study, we report for the first time the efficacy of *P. striatisporum* as a biological control agent of *Phytophthora* spp. In order to determine the mode of action, we examined the culture filtrates of the fungus for inhibitory effects against *P. capsici* Leonian. As this fungus is a slow colonizer, we tested the effect of an organic fertilizer, composted pig manure, on the colonization ability of Pst10 in the chilli pepper rhizosphere. The strain designated as Pst10 appeared to have high potential as a biocontrol agent of Phytophthora root rot of chilli in preliminary potting tests.

Application of Pst10 for soil-borne disease control of plant had been authorized and approved by the Chinese Invention Patent Bureau. The objectives of this research was to investigate the potential of Pst10 as a fungal biocontrol agent against PRR in chilli pepper.

2. Materials and methods

2.1. Fungal cultures and growth media

The isolate Pst10 of *P. striatisporum* was originally isolated from the rhizosphere of chilli pepper in an experimental greenhouse in Nanjing, China. Pst10 has been deposited at China General Microbiological Culture Collection Center and assigned Accession No. CGMCC No.1533.

The fungal cultures of Pst10 and pathogenic fungi, *P. capsici* Leonian, *Phytophthora infestans* (Mont.) de Bary, *Phytophthora drechsleri* Tucker, *Phytophthora nicotianae* Breda de Haan, *Phytophthora megasperma* Drechs. f. sp. medicaginis, *Botrytis cinerea* (De Bary) Whetzel, *Sclerotinia sclerotiorum* (*Lib.*)de Bary, *Rhizoctonia solani* J.G. Kühn, *Fusarium graminearum* Schwabe, *Fusarium oxysporum* Schltdl. and *Pythium aphanidermatum* (Edson) Fitzp., *Magnaporthe oryzae* B. Couch, *Alternaria solani* Sorauer, *Cladosporium cucumerium* Ellis et Arthur, *Saccharomyces cerevisiae* Meyen ex E.C. Hansen and *Candida albicans* (C.P. Robin) used in this study were maintained on potato-dextrose agar (PDA) slants at 4 °C and were grown on PDA plates at 25–26 °C in the dark.

2.2. Testing the antifungal spectrum of Pst10 using dual culture assays

The cultures of each of the test pathogens and Pst10 were grown on PDA plates at 25–26 °C for 5–7 days prior to the bioassay tests. The antifungal spectrum was determined by placing a 4-mm-diameter agar disk cut from the edge of an actively growing colony of Pst10 on one side of a PDA plate and an agar disk of the test pathogen of the same size on the opposite side of the same plate. For the yeasts, *S. cerevisiae* and *C. albicans*, cell suspensions obtained from 2-day-old cultures were spread around a Pst10 colony on PDA plate, respectively. Each combination was tested using five replicates. Zones of inhibition were determined after incubation in the dark at 25–26 °C for 8–10 days. The test was conducted twice.

2.3. Preparation of sterilized liquid culture filtrates (SLCF)

Erlenmeyer flasks (250 ml) containing 100 ml of potato-dextrose broth (PDB) were inoculated with four 8-mm-diameter mycelial disks obtained from the edge of vigorously growing colony of Pst10. Flasks were incubated in the dark at 25–26 °C on a rotary shaker at 160 rpm for 7 days. The liquid cultures were filtered through two-layers of cheesecloth in a funnel and the filtrates were centrifuged at 8000 rpm for 15 min. The supernatant was collected and sterilized by filtration through 0.22 μm Millipore membrane. The sterile filtrates were collected and stored at 4 °C before being used in the following experiment.

2.4. Toxicity of SLCF to P. capsici

2.4.1. Effect on mycelium growth of P. capsici

Four-mm-diameter mycelium disks of *P. capsici* were placed in the center of a series of PDA plates containing 2, 0.67, 0.4, 0.2, 0.1, 0.067, 0.05 ml of SLCF in 20 ml of molten PDA medium (45–50 °C) resulting in 10, 30, 50, 100, 200, 300, 400 fold dilutions, respectively. A 0-fold dilution treatment consisting of 20 μl SLCF was directly dropped onto each disk in plate. The control treatment was not amended with SLCF. Each treatment had five replicates. The plates were incubated in dark at 25–26 °C for 20, 48 and 120 h before colony diameters were measured. Disks of *P. capsici* which showed no growth after 7 days of incubation were transferred to PDA plates and incubated at 25–26 °C for 72 h to determine if they were dead or just inhibited. This experiment was conducted three times. Percent inhibition (%) was calculated as equal to the (colony diameter of control − colony diameter of treatment)/colony diameter of control × 100.

2.4.2. Effect on sporangial/spore formation or germination of P. capsici

Fifteen mycelial disks (8-mm-diameter) of *P. capsici* were placed in glass dishes (9 cm in diameter) containing 20 ml sterile water and 1 ml of sterilized filtrate of Pst10. Five replicates were used for each dilution and were grown for 24, 48, and 72 h at 25 °C with continuous light. The control treatment consisted of 15 disks of *P. capsici* (8 mm in diameter) placed in glass dishes (9 cm in diameter) containing only 20 ml sterile water. Sporangium for-