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Evaluation of *Streptomyces* spp. for the biological control of corky root of tomato and Verticillium wilt of eggplant

Giovanni Bubici*, Antonio Domenico Marsico, Margherita D'Amico, Mario Amenduni, Matteo Cirulli

Dipartimento di Scienze del Suolo della Pianta e degli Alimenti, Università degli studi di Bari 'Aldo Moro', via Amendola 165/A, 70126 Bari, Italy

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ABSTRACT

The effects of *Streptomyces* spp. isolates in the biological control of corky root of tomato and Verticillium wilt of eggplant was determined in *in vitro*, greenhouse and field trials. Twenty-six *Streptomyces* spp. isolates were obtained from the rhizospheres of different vegetable crops in southern Italy. In *in vitro* dual culture tests, mycelial radial growth of *Pyrenochaeta lycopersici* and *Verticillium dahliae* was reduced up to 18.6% and 30.1%, respectively. Radial growth of seven other fungal pathogens was variably reduced as well. The isolates StB-3, StB-6, StB-11 and StB-12 showed a good antagonistic effect against both *P. lycopersici* and *V. dahliae*, while the rest of isolates eventually showed antagonism against only one pathogen.

In pot-experiments in the greenhouse three of the four above-mentioned *Streptomyces* spp. isolates significantly reduced corky root up to 64.9% (StB-11), and all four isolates reduced foliar symptoms of Verticillium wilt (AUDPC) up to 48.3%, but none of them reduced the severity of vascular browning. In naturally infested field trials, StB-11 significantly reduced corky root severity in tomato by 48.2%, StB-12 by 35% and StB-6 by 32.6%, but none of the isolates were effective in controlling Verticillium wilt of eggplant. The effectiveness of the streptomycete AtB-42, successfully used in previous researches, was here confirmed as it reduced corky root of tomato in the field by 33.6%. In conclusion, our research demonstrates that under field conditions corky root of tomato, but not Verticillium wilt of eggplant, can be effectively controlled by the *Streptomyces* spp. isolates used in this study.

1. Introduction

After the phasing out of methyl bromide for soil fumigation in agriculture, the control of soil-borne plant pathogens has become a serious threat, in particular in those pathosystems lacking in resistant cultivars, such as corky root of tomato, caused by *Pyrenochaeta lycopersici* Schn. & Ger., and Verticillium wilt of eggplant, caused by *Verticillium dahliae* Kleb. (Polley, 1985; Ciccarese et al., 1994b). The currently allowed fumigants, such as metham-sodium, metham-potassium and dazomet, in general provide a lower control level of soil-borne pathogens compared to that achieved by methyl bromide (Martin, 2003). Several control measures for these two important diseases have been studied so far, including chemical, cultural and physical ones (reviewed by Bubici, 2006 and Bubici et al., 2006). Also, with the aim to find environmental-friendly control measures, several microorganisms have been widely studied as potential biological control agents (BCAs) of soil-borne pathogens (Paulitz and Bélanger, 2001). Information about biological control of *P. lycopersici* is limited to a few reports dealing with the use of *Trichoderma* spp. Pers., *Bacillus subtilis* (Ehrenberg) Cohn and *Streptomyces* spp. Waksman & Henrici (Whipps, 1987; Vanachter et al., 1988; Ciccarese et al., 1994a; Colella et al., 2001; Perez et al., 2002). In contrast, more efforts have been dedicated for searching BCAs against *V. dahliae*, including fungi (mainly *Gliocladium* spp. Corda, *Talaromyces flavus* (Klöcker) Stolk & Samson, and *Trichoderma* spp.), and bacteria [mainly *Bacillus* spp., *Paenibacillus alvei* Cheshire and Cheyne, *Pseudomonas* spp. Migula, *Serratia plymuthica* (Lehmann and Neumann) Breed et al., and *Streptomyces* spp.] (for reviews see Baker, 1981; Pegg and Brady, 2002; Bubici and Cirulli, 2008, 2011). Many of these BCAs were isolated from suppressive soils and compost substrates (Berg et al., 2001, 2005; Tjamos et al., 2004). Also, mycorrhizal, fungal and bacterial endophytes have been studied as BCAs against *V. dahliae* (Narisawa et al., 2002; Tjamos et al., 2004; Goicoechea et al., 2010).

Streptomycetes are recognized as good producers of several secondary metabolites, antibiotics and lytic enzymes affecting the growth of fungal pathogens (Doumbou et al., 2001). Members of this family are consistently referred in the literature as potential

* Corresponding author. Present address: Istituto di Virologia Vegetale, Consiglio Nazionale delle Ricerche (CNR), via Amendola 165/A, 70126 Bari, Italy.
Tel.: +39 080 5443109; fax: +39 02700414517.
E-mail addresses: g.bubici@ba.ivv.cnr.it, giovabubi@email.it (G. Bubici).

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BCAs against a large number of soil-borne pathogens, including *P. lycopersici* and *V. dahliae* (Chi, 1963; Azad et al., 1987; El-Abyad et al., 1993, 1996; El-Quakfaoui et al., 1995; Aghighi et al., 2004). Also, proliferation of streptomycetes has been associated with natural suppressiveness of soil, as well as other cropping substrates such as rockwool, against several soil-borne fungi, including *Pythium aphanidermatum* (Edson) Fitzp., *Rhizoctonia* spp. DC. and *Streptomyces scabies* (Lambert and Loria) (Lorang et al., 1995; Liu et al., 1996; Ryan and Kinkel, 1997; Postma et al., 2005; Mazzola, 2002; Mazzola et al., 2007). The *in vitro* antimicrobial activity of streptomycetes is due to molecules, such as antibiotics and extracellular enzymes, diffused through the growth medium (Goodfellow and Williams, 1983; Korn-Wendisch and Kutzner, 1999). Several studies have elucidated some antibiotics involved in antifungal activity, and nigericin, geldamycin, a complex of macrocyclic lactone antibiotics, 1-propanone 1-(4-chlorophenyl) and strevertenes are just some examples (Rothrock and Gottlieb, 1984; Valois et al., 1996; Trejo-Estrada et al., 1998; Beauséjour et al., 2003; Ezziyyani et al., 2007; Kim et al., 2011). Other studies have demonstrated the role of chitinase and β-1,3-gluacanase in the antifungal activity (Singh et al., 1999; Prapagdee et al., 2008). Streptomycetes may act also by the fumigant activity of volatile compounds such as geosmin (Wan et al., 2008; Li et al., 2010).

Presently, several bio-fungicides for the control of soil-borne diseases are available as commercial products worldwide (Paulitz and Bélanger, 2001). In Italy, a few commercialized products specifically registered as biological fungicides for the control of soil-borne diseases contain *Coniothyrium minitans* W.A. Campb., species of *Trichoderma*, or *Streptomyces griseoviridis* Anderson strain *K61*, while other products are marketed as plant growth promoters, plant strengtheners, or soil conditioners. All these products are reported to target at several soil-borne pathogens, such as *Fusarium* Link, *Phytophthora* de Bary, *Pythium* Pringsheim, *Rhizoctonia*, *Sclerotinia* Fuckel, *Thielaviopsis* Went and others, but usually not at *P. lycopersici* or *V. dahliae* are not usually referred to as target pathogens. Informations are scarce on the control by streptomycetes of soil-borne diseases in the field.

Therefore, the research reported here was aimed at screening *Streptomyces* isolates for their effectiveness in controlling corky root of tomato and Verticillium wilt of eggplant. *Streptomyces* isolates were obtained from the rhizosphere of vegetable crops, and evaluated for: (a) their antagonistic activity *in vitro* against *P. lycopersici* and *V. dahliae*, (b) their effectiveness in the control of the diseases in the greenhouse, and (c) effectiveness of selected promising *Streptomyces* isolates for biological control of the diseases in the field.

2. Materials and methods

2.1. Isolation of streptomycetes

Streptomycetes were isolated from the rhizosphere of vegetable crops in southern Italy, and from plots of one experimental field at Valenzano, Bari, Italy, where diverse soil treatments had been applied before the last cropping (Table 1).

From each locality, 1 kg soil samples, each obtained by mixing five 200 g sub-samples, were taken at 5–20 cm depth from an area of at least 1000 m^2. Isolation of streptomycetes from soil was carried out by the serial dilution method. Soil samples were air dried for 15 days and passed through a 2 mm sieve. Ten grams of air-dried soil were suspended in 100 mL of a sterile physiological solution consisting of 8.5 g NaCl, 1 g agar, two drops of Tween® 20 (Sigma Aldrich) and 1 L distilled water. After a 30 min stirring on a magnetic agitator, 2 mL of this suspension were then suspended in a vial containing 18 mL of the physiological solution and hand-shaken. Serial dilutions down to 10^{-7} were made. One-hundred microliters of suspension from each dilution from 10^{-2} to 10^{-7} were spread out with the aid of a glass rod onto 9 mm Petri plates containing the semi-selective medium of Küster and Williams (1964) amended after sterilization with 0.05 g L^{-1} of cycloheximide sulphate (Williams and Cross, 1971). Three plates (replications) were made per dilution. Plates were incubated in the dark at 24 °C for 7 days. *Streptomyces* colonies were visually identified by their morphological macroscopic characteristics (Shirling and Gottlieb, 1966; Korn-Wendisch and Kutzner, 1999) and singly transferred by streaking onto potato-sucrose-agar (PSA; 200 g L^{-1} pealed potato, 20 g L^{-1} sucrose, 15 g L^{-1} agar). Single-spore colonies were generated by one sub-culturing and stored at 4 °C in glass tubes provided with cotton plug and containing PSA. A total of 26 *Streptomyces* isolates were obtained from the soil samples (Table 1).

The streptomycete AtB-42 was also included in the *in vitro*, greenhouse and field tests as a reference isolate. It was isolated from soil, and tested *in vitro*, in the greenhouse and field against *P. lycopersici* and *V. dahliae*. In particular, AtB-42, applied in the field in mixture with an olive husk compost, provided 30% tomato corky root reduction and increased tomato yield by 30%, but did not controlled Verticillium wilt of artichoke (Colella et al., 2001).

2.2. In vitro antagonistic activity of streptomycetes

The antagonistic activity of the 26 *Streptomyces* isolates was tested *in vitro* against *P. lycopersici* (Ply-1 obtained from tomato at Valenzano, Bari, Italy) and a non-defoliating *V. dahliae* isolate (Vd270, obtained from eggplant at Valenzano, Bari, Italy). In addition, the following seven fungal pathogens were also included in order to evaluate the antifungal spectrum of the *Streptomyces* isolates: *Alternaria alternata* (Fr.) Keissl., isolated from kiwi tree leaf, *Botrytis cinerea* Pers., isolated from tomato stem, *Cylindrocarpon destructans* (Zinssm.) Scholten, isolated from strawberry roots, *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) W.C. Snyder & H.N. Hansen, isolated from tomato stem, *Phellinus torulosus* (Pers.) Bourdot & Galzin, isolated from peach trunk, *Rhizoctonia solani* Kühn, isolated from tomato roots, and *Sclerotinia sclerotiorum* (Lib.) de Bary, isolated from lettuce leaf.

The streptomycete inoculum was prepared as follows: a suspension was prepared by washing with sterile distilled water 14-day colonies grown on PSA and obtained by sub-culturing the collection glass tubes. Spore concentration was estimated using a haemocytometer and adjusted by diluting with sterile distilled water. The pathogen inoculum consisted of fungus-colonized agar disks taken with a corky borer from the edge of 7 days old colonies grown on PSA. The *in vitro* assays were carried out by the dual culture method using 90 mm-Petri dishes containing PSA. Twenty microliters of a spore suspension (5×10^7 spores mL^{-1}) of each streptomycete isolate were streaked along a diameter of the plate as a linear culture, and two agar disks (6 mm diameter), were removed orthogonal to and equidistant from the streptomycete streak and the plate edge, and replaced with *P. lycopersici*- or *V. dahliae*-colonized disks). Plates inoculated with the pathogen and without the streptomycete were used as negative controls. Six replicates (plates) were used for each treatment combination, as for a completely randomized design. The plates were incubated at 25 °C in the dark, and the diameter of the pathogen colonies, orthogonal to the streptomycete streak, was measured after 14 days incubation.

2.3. Disease control trials in greenhouse

The 26 *Streptomyces* isolates and the reference isolate AtB-42, were evaluated for the control of corky root of tomato and Verticillium wilt of eggplant in two consecutively repeated greenhouse trials. Forty-five day-old seedlings of tomato cv. Diaz and eggplant cv. Mission Bell, both highly susceptible to their respective