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SHORT COMMUNICATION

Biological control of tomato bacterial spot by seed microbiolization

Carine R. Naue, Dediel J. A. Rocha & Andréa B. Moura

Departmento de Fitossanidade, Universidade Federal de Pelotas, 96010-970, Pelotas, Rio Grande do Sul, RS, Brazil

Author for correspondence: Andréa B. Moura, e-mail: abmoura@ufpel.edu.br

ABSTRACT

Six plant growth promoting rhizobacteria isolates - two *Streptomyces* (DFs1296 and DFs1315), three *Bacillus* (DFs1414, DFs1420 and DFs1423) and one *Pseudomonas* (DFs1421) - were used to microbiolize tomato seeds for the control of bacterial spot caused by *Xanthomonas gardneri*. Three assays were conducted in a completely randomized design with six replications. In each assay, *X. gardneri* suspensions (10^8CFUmL^{-1}) were spray-inoculated on the leaves. In the first and second assays, three and four leaves, respectively, were assessed for disease severity. In the third assay, three leaves per plant were assessed for the number of lesions per leaf, leaf area and dry weight. Results showed that one of the *Bacillus* sp. isolate (DFs1420) consistently provided the greatest relative reduction (48%) of tomato bacterial spot severity.

Key words: *Solanum lycopersicum, Xanthomonas gardneri*, rhizobacteria, PGPR, biocontrol.

Tomato bacterial spot, caused by *Xanthomonas* species, is of widespread occurrence in Brazil and may cause losses of up to 35% in the yield (Vale et al*.,* 2004). Losses have been reported in tomato crops cultivated both for fresh market and for industrial processing, especially during warm (>25ºC) cropping conditions (Lopes & Quezado-Soares, 1997). Eggplants and bell peppers are other economically important hosts for this pathogen (Lopes & Quezado-Soares, 1997). This disease occurs in all aerial tissues of the plant, causing flowers and forming fruits to fall. Symptoms on the leaves are brown circular spots spread on the leaf surface. Circular lesions with slightly raised edges and a corticated aspect are observed on fruits (Kurozawa & Pavan, 2005).

To reduce losses caused by bacteria, integrated practices are recomeneded such as the use pathogen-free seeds and seedlings, roguing, crop rotation, use of less susceptible cultivars and chemical treatment (Lopes & Quezado-Soares, 1997). However, the continuous use of antibiotics, such as streptomycin and oxytetracycline, and copper fungicides has led to the emergence of *Xanthomonas* spp. populations that are resistant to these products (Quezado-Duval et al*.,* 2003), in addition to food and environment contamination and human and animal poisoning (Bettiol & Morandi, 2009).

Given the need for alternatives to chemicals that are less harmful to the environment, the development of biological products for the control plant pathogens is being encouraged. Rhizobacteria are among microorganisms used for such a purpose because they can be both antagonists for plant pathogens and promoters of plant growth and yield increase.

Rhizobacteria of the genera *Pseudomonas* and *Bacillus* have attractive features for use in biological control programs (Noronha et al., 1995). The potential of these microorganisms as antagonists has been observed in several biological control studies of diseases affecting different crops (Bettiol & Morandi, 2009). In tomatoes, the control of bacterial diseases using rhizobacteria has been tested with success, reaching 100% control in some cases, under controlled conditions and over a short period of time (Moura et al*.,* 1998; Aysan et al*.,* 2003; Tan et al., 2006; Moss et al*.,* 2007). The present study aimed to evaluate the effects of treatment of tomato seeds with rhizobacteria on the control of tomato bacterial spot.

Six rhizobacteria isolates were involved, namely: two *Streptomyces* spp. (DFs 1296 and DFs1315) (Moura, 1996); three *Bacillus* spp. (DFs1414, DFs1420 and DFs 1423) and one *Pseudomonas* sp. (DFs1421) (Deuner, 2004). These bacteria were identified through sequencing of rDNA 16S gene portion at the Centro de Genômica e Melhoramento of the Universidade Federal de Pelotas (Pelotas, RS, Brazil). Sequencing was performed using the Megabace 1000 (Gene ID). The nucleotide sequence was aligned and compared with those deposited in the NCBI database using the ClustalW program. The sequences were deposited in GenBank and were given the access numbers KC815306 and KC815307 for the streptomycetes and KC815303, KC815304, KC815305 and KC815308 for the remaining

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rhizobacteria.The pathogenic isolate used was provided by the Laboratório de Bacteriologia Universidade Federal de Viçosa (UFV). The species was determined through rDNA 16S sequencing in the Laboratório de Genômica (Bioagro, UFV, Viçosa, MG, Brazil). Sequencing was performed as previously described. The sequenced fragment allowed identification of the *Xanthomonas* isolate as *X. gardneri* (data not shown).

Three assays were conducted in this study. For this purpose, tomato seeds of the SuperMarmande cultivar (Gaúcho/Maçã) were kept immersed for 4 h with stirring at 4°C in a cell suspension (10^8 CFU.mL^{-1}) of each rhizobacteria in a saline solution (0.85% NaCl) with 24h growth in a Kado & Heskett medium (1970) (A_{540}= 0.50). Seeds immersed in saline solution served as a control. After the period of exposure of seeds to rhizobacteria, five seeds were immediately deposited in plastic cups (700 mL) containing Plantmax® substrate. After germination, only one seedling was kept per cup, forming an experimental unit. The plants were sprayed with a cell suspension of the pathogen (10^8 CFUmL^{-1}) 30 days after sowing, and they were kept in a moist chamber for 24 h before and 48 h after inoculation.

For the first two assays, evaluations were performed 12 days after inoculation (onset of symptoms). In the first and second assays, three and four leaves were assessed for severity, respectively, with the aid of a diagrammatic scale (Melo et al. ,1997). For the third assay, the number of lesions per leaf was determined on three leaves per plant at 13, 15, 17 and 19 days after inoculation. These values were used to calculate the area under the number of lesions progress curve (AULPC) using specific software (Maffia, 1995). In the third assay, the leaf area of the three leaves was estimated using LI-3100 area meter (LI-COR). Subsequently, the leaves were kept in a drying oven at 60°C for three days, and then weighed to determine their mass.

The assays were arranged in a completely randomized design with six replications (portions of a plant). Data from the AULPC obtained in the third assay were transformed into $\sqrt{x+0,5}$ and subjected to analysis of variance. The means were compared using Tukey's test at 5% using Winstat statistical software (Machado & Conceição, 2007). For the other variables (dry weight and foliar area) and for data obtained in the first and second assays, original data (non-transformed) were used.

The overall mean (three assays) percent control of tomato bacterial spot for DFs1420 was 48% and yielded a consistent result in contrast with the other isolates evaluated (Table 1). In the first assay, DFs1296 and DFs1420 controlled bacterial spot in 60% which was the best performance among the six rhizobacteria tested. In the second assay, only the isolate DFs1420 was efficient in reducing the disease (39%). In the third assay, the DFs1420 isolate also provided the greatest disease reduction (50%) with the lowest AULPC value, promoting the growth of the plants and leading to greater dry weight (Table 1).

The percentages of disease control obtained in the present study were higher than those found by Byrne et al. (2005) and Kavitha & Umesha (2007) for the same pathosystem. These authors reported 36.4% disease control using *Pseudomonas syringae* and 20 to 30% using *P. fluorescens,* respectively. Conversely, inconsistent result, such as that observed for DFs1296 (only effective in the first assay), is not uncommon and can be attributed to several factors (Ludwig & Moura, 2007).

Several studies have shown both biocontrol and growth promotion effect induced by the same bacterial isolate, such as for DFs1420. Growth-promoting rhizobacteria are also known to effectively control fungal tomato diseases, such as Fusarium wilts and seedling damping-off caused by *Rhizoctonia* (Domenech et al., 2006). Bacterial diseases that affect tomato, such as *Ralstonia solanacearum*, were controlled by growth promoters such as actinomycetes (El-Abyad et al., 1993; Moura & Romeiro, 2000) and *Pseudomonas* (Peixoto et al., 1995). Recently, Kavitha & Umesha (2007) showed that growth-promoting

TABLE 1– Severity (percent diseased leaf area) and area under the number of lesion progress curve (AULPC), dry weight and leaf area of tomato originating from microbiolized seeds with different rhizobacteria, inoculated with *Xanthomonas gardneri* in the greenhouse.

Treatment	Assays				
	First	Second	Third		
	Severity*		AULPC**	Dry weight (g)	Leaf area (cm^2)
Control	38.9a	32.2a	29.5ab	0.303abc	14.4bc
DFs1420	16.6b	20.9b	15.1c	0.425a	18.8a
DFs1423	39.4a	27.8ab	21.7abc	0.406ab	16.1bc
DFs1421	26.1b	28.4ab	18.0bc	0.273bc	12.6bc
DFs1414	32.9a	33.6a	26.1abc	0.273bc	11.7cd
DFs1296	16.6b	24.2ab	35.7a	0.185c	8.3d
DFs1315	32.8a	29.4ab	17.3bc	0.300bc	13.3bc
CV (%)	29.0	18.2	61.1	25.3	25.5

Means followed by different letters in the column differ significantly by Tukey's test (α=0.05).
*percent diseased leaf area estimated with the aid of a diagrammatic scale (Melo et al., 1997). ** Area under the number of lesion progress curve, based on the number of lesions per leaf evaluated at 13, 15, 17 and 19 days after inoculation.

Pseudomonas also controlled bacterial diseases of the shoot, such as bacterial spot caused by *Xanthomonas vesicatoria.*

The variability in the efficacy of control of bacterial spot for the isolates used in this work (except for DFs1420) was previously registeded by Ludwig & Moura (2007) and it was associated with the stage of plant development, geographical areas (Byrne et al*.,* 2005) or cultivars (Kavitha & Umesha, 2007). The stability of the control by the microorganism is usually associated with the performance of multiple mechanisms of action (Guetsky et al*.,* 2001) and with an aggressive colonization of the tissues of the host plant (Kloepper & Beauchamp, 1992). In the present study, the mechanisms of action of the DFs1420 isolate were not studied here but some of its capabilities are already known. The DFs1420 rhizobacteria, previously known as B403R, colonizes roots of different tomato cultivars and has an inhibitory capacity due to production of *in vitro* antibiotics against *Alternaria solani* (Ell & Mart), *R. solanacearum*, *Rhizoctonia solani* (Kuhn)*, Sclerotinia sclerotiorum* (Lib.) De Bary and *Stemphylium solani* (Weber) (Deuner et al., 2004). Considering the promising results obtained for DFs1420 in this work, its efficiency in controlling bacterial spot should next be evaluated in the field.

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