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ANTAGONISM OF *SERRATIA MARCESCENS* TOWARDS *PHYTOPHTHORA PARASITICA* AND ITS EFFECTS IN PROMOTING THE GROWTH OF CITRUS

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SHORT COMMUNICATION

ABSTRACT

Phytophthora parasitica causes serious widespread, and difficult–to–control root rots in warmer regions. This oomycete is one of the most important pathogen of citrus. This paper reports the biological control of the pathogen by a strain of *Serratia marcescens* R-35, isolated from citrus rhizosphere. In greenhouse trials, the bacterium suppressed more than 50% of the disease and promoted the plant growth.

Key words: biological control, rhizobacteria, plant growth promotion

Phytophthora parasitica infects many citrus species, causing a widespread disease known as gummosis. This is a prevalent disease in São Paulo State, Brazil. Infected trees generally lack vigor and may die prematurely. The disease has became increasingly important in citrus commercial orchards and nurseries and there is a need for alternate disease management. The use of microorganisms as a means of biological control for this disease is of special interest. Chemical control provided by highly effective Oomycetes-specific fungicides, such as metalaxyl and fosetyl Al, has been successful (2), but is not always desirable because of the high cost of application, potential hazards to the environment, and the development of fungicide-resistant strains. Some rhizobacteria have been reported to be good antagonists of *Phytophthora* spp. (8,9). Most studies have emphasized bacterial agents such as *Pseudomonas* spp., *Agrobacterium* sp., *Bacillus subtilis* and *Serratia marcescens*.

The use of plant-growth-promoting rhizobacteria as inoculants depends on their ability to colonize the root system and to compete with indigenous microflora (6). Efficient colonization of roots may present further establishment of pathogens by both physical and nutritional competition (10). Colonization is initiated by the process of bacterial attachment to roots, where such competition phenomena are already operating. We report here the isolation of a *Serratia marcescens* strain isolated from citrus rhizosphere in São Paulo State and its antagonistic activity *in vitro* and *in vivo* against *Phytophthora parasitica*.

Serratia marcescens strain R3.5 was isolated from washed root surface of healthy plants of commercial crops of citrus. It was obtained following plating of serial dilutions of sample material onto king's B medium (5). Pure culture of *S. marcescens* was characterized by analysis of 16S rDNA gene.

Antagonism of *S. marcescens* towards *P. parasitica* was tested in a dual–plate assay on PDA (potato-dextrose-agar) and KBM, (5) following the following procedure. One disk (6 mm in diameter) of *P. parasitica* was inoculated equidistantly (60 mm) on PDA and KBM from *S. marcescens*. It was used four replicates per treatment and the antagonistic activity was measured after 7 days. The level of inhibition was defined as the subtraction of the fungal growth radius of a control culture from the distance of the growth in the direction of *S. marcescens*. The fungicide metalaxyl was used as positive control. Microscopic observation were done without any citochemical method regions of the interactions between the antagonist and the pathogen were cut off and put in on

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microscope slides and viewed directly in optic microscope using immersion lens.

S. marcescens was tested for plant-growth-promoting activity and control of *P. parasitica* towards citrus (*C. limonia*) in greenhouse trials. As substrate it was used a commercial mixture Plantmax. The mixture was sterilized by autoclaving for two cycles at 127ºC. Thirty-day-old-citrus sterile seedlings were transferred to pots (20 cm diameter, with four plants per pot and five pots per treatment in randomized complete blocks), and each plant was then inoculated by dipping with approximately 10^8 UFC.mL^{-1} viable cells of *S. marcescens*. Half of the pots were previously inoculated with *P. parasitica*. *Phytophthora*-colonized wheat seeds (2g) were added to each pot. The incidence of the disease was based in a index on a scale of 0-4, where: 0 healthy plants and 4 dead plant. The experiments carried out in greenhouse conditions were done twice, with similar results.

The *in vitro* antagonism assays showed that the strain R3.5 of *S. marcescens* is an antagonist to *P. parasitica*. *S. marcescens* showed the highest activity against *P. parasitica* in KB medium when compared to PDA medium reducing the mycelial growth in 40% in KB and 37% in PDA. The inhibition of fungal growth in KB (a Fe (III) – poor medium) is likely to be mediated by production of siderophores besides antibiotics (4). The fungicide metalaxyl, used to control plant pathogens from the Peronosporales order, completely inhibited the mycelial growth of *P. parasitica*. The bacterium exhibited an antagonistic activity through antibiosis. Micelium of *P. parasitica* appeared red due the red pigments produced by *S. marcescens* (Figure 1) and, when in contact to *Phytophthora*, *Serratia* lysed the oosporos of the pathogen. The oospore germination of *P. parasitica* was affected by the culture filtrate of *S. marcescens* (data not shown).

In test for PGPR activity, *S. marcescens* resulted in increased growth (in terms of dry weight of root and shoot) (Table 1). The bacterium reduced the seedling infection in 50%. It was not investigated the mechanism by which *S. marcescens* biologically control the gummosis in citrus. Biocontrol of a pathogen generally has been attributed to one individual compound or mechanism, though the roles of other possible factors have been discussed. *Phytophthora* species have been biologically controlled by different rhizobacteria, including *Brevibacterium linens*, *Bacillus thuringiensis* and *Bacillus subtilis* active against *P. vignae* (3); *Pseudomonas vignae* active against *P. citrophthora* and *P. parasitica* (1); and *Enterobacter aerogenes* against *P. cactorum* (7).

Evidence to date has shown that *S. marcescens* is capable of inducing plant growth and suppresses *P. parasitica*, and also to establish in the rhizosphere. Thus, all these criteria make it a potential biocontrol agent of gummosis disease.

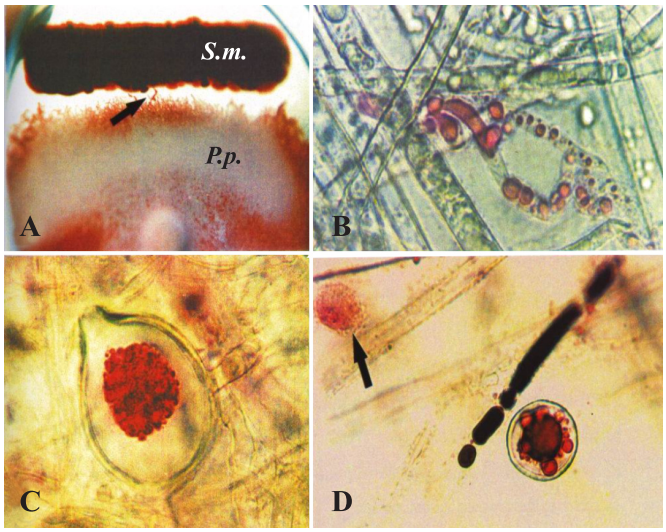

Figure 1. *In vitro* activity of *Serratia marcescens* (R3.5) against *Phytophthora parasitica*. (A) Inhibition of *P. parasitica* (Pp) by *S. marcescens* (Sm); (B) Metabolites of *S. marcescens* (in red) inside hyphae of *P. parasitica*; (C) Metabolite of the bacterium inside sporangia; (D) Lysis of hyphae and sporangia by metabolites of *S. marcescens*. Magnification of 1000 times.

Table 1. Disease incidence in citrus seedlings treated with *Serratia marcescens* in substrate artificially infested with *P. parasitica* and the effects of the bacterium on shoot and root dry weight of *Citrus limonia*.

Treatment	Incidence root rot	Plant height of (cm)	Shoot dry weight (g)	Root dry weight (g)
S. marcescens	2 b	35.0 b	2.3 a	0.52 b
Metalaxyl	2 b	36.6 b	2.7 a	0.65 b
Control (*P. parasitica*)	4 a	25.3 a	2.0 a	0.30 a

Values are the means of there replicates. Values designated with the same letter are not significantly different (P = 0.05) according to these. Incidence of disease is based in a index on a scale of 0 - 4: 0 healthy plants (no disease) and 4 plant dead. 4: dead plants, or more than 50% of the root system rotted.

RESUMO

Antagonismo de *Serratia marcescens* contra *Phytophthora parasitica* e seu efeito na promoção do crescimentos de citros

Phytophthora parasitica é um oomiceto que causa sérios problemas fitossanitários em diferentes espécies de plantas em regiões tropicais e o controle tem sido difícil. Este patógeno é um dos mais importante à citricultura. Este trabalho relata o

controle biológico do patógeno por uma linhagem de *Serratia marcescens* R-35, isolada da rizosfera de citros. Em condições de casa-de-vegetação, a bactéria reduziu em mais de 50% a incidência da doença, ao mesmo tempo que promoveu o crescimento de plantas.

Palavras-chave: controle biológico, rizobactérias, promoção de crescimento

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