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Control potential of *Meloidogyne javanica* and *Ditylenchus* spp. using *fluorescent Pseudomonas* and *Bacillus* spp.

Marcielly F. Turatto[a], *Fernanda dos S. Dourado*[b], *Jerri E. Zilli*[b], *Glória R. Botelho*[c,*]

[a] *UFSC Centro Curitibanos, Rod. Ullisses Gaboardi s/n, km.03, CEP 89520-000, Curitibanos, SC, Brazil*
[b] *Embrapa – Agrobiologia, Rodovia BR-465, Km 7 (antiga Rodovia Rio/São Paulo), CEP 23891-000, Seropédica, RJ, Brazil*
[c] *UFSC Centro Curitibanos, Rod. Ullisses Gaboardi s/n, km.03, CEP 89520-000, Curitibanos, SC, Brazil*

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ABSTRACT

Plant Growth Promoting Rhizobacteria (PGPR) have different mechanisms of action in the development of plants, such as growth promotion, production of phytohormones and antibiotic substances and changes in root exudates. These help to control plant diseases. In order to evaluate the potential of microorganisms in the control of *Meloidogyne javanica* and *Ditylenchus* spp., five rhizobacteria isolated from rhizosphere of garlic cultivated in the Curitibanos (SC) region were tested. Hatching chambers were set on Petri dishes, in which were added 10 mL of bacterial suspension and 1 mL of *M. javanica* eggs suspension, at the rate of 4500, on the filter paper of each chamber. The same procedure was performed with 300 juvenile *Ditylenchus* spp. The experimental design was completely randomized, with four replications. The evaluations were performed every 72 h for nine days. The antagonized population of nematodes was determined in Peters counting chamber, determining the percentage hatching (for *M. javanica*) and motility (for *Ditylenchus* spp). Isolates CBSAL02 and CBSAL05 significantly reduced the hatching of *M. javanica* eggs (74% and 54.77%, respectively) and the motility of *Ditylenchus* spp. (55.19% and 53.53%, respectively) *in vitro*. Isolates were identified as belonging to the genera *Pseudomonas* (CBSAL05) and *Bacillus* (CBSAL02).

Introduction

Some of the plant crops commodity cultivated in Curitibanos region (SC) are garlic (*Allium sativum*) and soybean (*Glycine max* (L) Merril.). Those two crops are used in the main rotation system adopted by local farmers. In the case of garlic production, Curitibanos ranks as the leading producer in the state of Santa Catarina. Soybean production in the region is low; however, the genetic potential of the crop generates a secure market and serves as an alternative crop to increase the income of the producer.

As with all crops, garlic and soybeans are also susceptible to a variety of pests and diseases. Among biotic factors,

* *Corresponding author at*: UFSC Centro Curitibanos, Rod. Ullisses Gaboardi s/n, km.03, CEP 89520-000, Curitibanos, SC, Brazil.
E-mail: gloria.botelho@ufsc.br (G.R. Botelho).

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nematodes cause significant drop in production. These organisms parasitize both the roots and the aerial parts of the plants.

The main nematode parasite in garlic is *Ditylenchus dipsaci*, which is distributed in all production regions of Brazil.[1] One of the factors hindering its control is the anhydrobiosis ability of the fourth instar juvenile.[2] In addition, few chemicals are released by the Ministry of Agriculture Livestock and Food Supply to control this agricultural pest.[3]

On the other hand, the nematodes of the genus *Meloidogyne* cause the most damage to soybean.[4] Due to monoculture or rotation with plant species that are also the host, coupled with low ground cover, the genus finds a favorable environment for its development.

Currently, control of nematodes is accomplished through the use of nematicides that are high-priced products and have residual action, as well as high toxicity to the environment and soil microorganisms.[5] Because of the possible negative impacts that it can cause[6] and restrictions in the use of these products, biological control can become a great ally in the control of these plant parasites.[7]

An alternative to chemical control is the use of antagonist microorganisms. The Plant Growth Promoting Rhizobacteria (PGPR) can be powerful agents for biological control of plant parasitic nematodes. *Bacillus* and fluorescent *Pseudomonas* group are among the most studied and with the highest correlation to soil suppressiveness.[8] Rhizobacteria have several mechanisms of action for the plant disease control.[9] The main mode of action of this group of bacteria is through the production of enzymes, antibiotics, siderophores, changes in root exudates, and induced resistance, among others.[10] According to Becker et al.,[11] changes in root exudates can inhibit the hatching of eggs in nematodes, or even reduce the attractiveness of these to the roots of plants. Freitas et al.[12] confirmed the potential of *Pseudomonas fluorescens* isolates to control nematodes by achieving a 75% control of *Heterodera schachtii*. The same isolates also controlled *Meloidogyne* spp. and *Radopholus similis* in maize, banana and tomato. Isolates of the same bacterial species have been reported as effective in the inhibition of *M. incognita* on tobacco, when inoculated into the soil with *Trichoderma harzianum*, and the action resembles the nematicides Furadan and phorate.[13]

In this paper, the objective was to evaluate the effect of rhizobacteria, belonging to Rhizobacteria collection of Laboratório de Microbiologia da UFSC Centro de Curitibanos, in the control of *Meloidogyne javanica* and *Ditylenchus* spp.

Materials and methods

Effect of rhizobacteria isolates in the hatching of eggs of M. javanica in vitro

Five isolates of rhizobacteria (CBSAL02, CBSAL05, CBSAL14, CBSAL18 and CBSAL21) from Rhizobacteria Collection of the Laboratório de Microbiologia from Universidade Federal de Santa Catarina Centro Curitibanos were selected. The rhizobacteria were grown in Petri dishes containing solid King B medium and subsequently incubated for 24 h at 28 °C. After growth, the suspension of these isolates was held in sterile distilled water with the aid of Drigalski strap, under aseptic conditions. Each suspension was placed into sterile glass bottles. The optical density of the bacterial suspensions was adjusted to 0.2 optical density (wavelength 625 nm).

The eggs of *M. javanica* used in the experiment were provided by Dr. Bruno Barbosa Unesp Jaboticabal (SP). Hatching chambers were assembled according to the methodology proposed by Alves et al.,[14] in which were added 10 mL of each bacterial suspension. Then, onto the filter paper was added 1 mL of the egg suspension of *M. javanica*, containing approximately 4500 eggs. As a control treatment, only sterile water with *M. javanica* eggs was used. The chambers were conditioned at a temperature of 29 °C for a period of nine days. The statistical design was completely randomized, with six treatments and four replications, each of which has a hatching chamber. For every 72 h (three days), aliquots were removed from the suspensions of the chambers for evaluation of the degree of inhibition of egg hatching by each bacterial suspension.

The population of nematodes hatched, which was retained on the filter paper, was determined with the aid of an optical microscope and Peters counting chamber. Nematodes recovered below the paper were recorded as not antagonized. For counting was determined the hatched individuals in the control (100% hatching) and then, in the other treatments. The percentage of hatching was calculated by comparison with the number in the control. The volume of bacterial suspension removed was replaced individually in each chamber, after the withdrawal according to each treatment.

Effects of rhizobacteria isolates on the motility of Ditylenchus spp.

The preparation of the inoculum followed the same methodology described above.

The population of nematodes was extracted from garlic plants contaminated by the nematode samples. The methodology used was flotation–centrifugation in sucrose solution with kaolin.[15] After extraction, individual nematodes were placed in Petri dishes containing 20% formaldehyde. Later, the nematodes were identified with the help of the identification key proposed by Mekete et al.[16] After being identified as belonging to *Ditylenchus* genus, the population was established as 300 nematodes/mL in the extracted suspension, regardless of the stage of development of the individual.

Hatching chambers were prepared and to it 10 mL of each bacterial suspension was added. Then, to the filter paper, 1 mL of *Ditylenchus* spp. suspension containing approximately 250 individuals, at different stages of development, was added. As a control treatment, only sterile water added to the filter paper with nematodes was used. The chambers were placed in 29 °C, for a period of nine days. The statistical design was completely randomized, with six treatments and four replications, with each plot having a hatching chamber.

At intervals of 72 h, samples were taken from the suspensions in the chambers to evaluate the degree of antagonism of each isolate.

The population of antagonized nematodes retained on the filter paper was determined with the aid of an optical

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