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SHORT COMMUNICATION

Selection of rhizobacteria able to produce metabolites active against *Meloidogyne exigua*

Denilson F. Oliveira · Vicente P. Campos · Daniel R. Amaral · Alexandro S. Nunes · José A. Pantaleão · Deivid A. Costa

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Abstract This work aimed to select potentially useful rhizobacteria for the control of *Meloidogyne exigua*, a widely disseminated root-knot nematode found in Brazilian coffee fields. Initially, crude metabolites produced in liquid medium by 69 isolated rhizobacteria strains (previously obtained from several plants collected in the south of Minas Gerais State, Brazil) were tested in vitro for lethality towards *M. exigua* second stage juveniles (J_2). Substances produced by strains of *Bacillus cereus, B. megaterium, B. pumilus, B. thuringiensis, Enterobacter asburiae, E. cloacae* and *Paenibacillus macerans* caused high mortality in J_2. Subsequently, the ten most active crude metabolites were selected and assessed for nematicidal activity in a biotest with *M. exigua*-inoculated coffee plants. Although strains of *B. cereus, B. pumilus* and *P. macerans* caused a significant reduction in the number of root galls and/or nematode eggs per plant 90 days after treatment, the best results were obtained with the application of *B. megaterium* strain 54-06.

D. F. Oliveira (✉) · A. S. Nunes · J. A. Pantaleão · D. A. Costa
Departamento de Química, Universidade Federal de Lavras, C.P. 3037, CEP 37.200-000 Lavras, MG, Brazil
e-mail: denilson@ufla.br

V. P. Campos · D. R. Amaral
Departamento de Fitopatologia, Universidade Federal de Lavras, C.P. 3037, CEP 37.200-000 Lavras, MG, Brazil

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Root-knot nematodes (*Meloidogyne* spp.) cause losses as high as US$ 400 mi/yr to coffee producers in Brazil (Santos 2000). The use of rhizobacteria that can produce nematicidal metabolites is considered a promising tool for controlling these nematodes (Campos et al. 1998; Ali et al. 2002).

In our study, initially we screened crude metabolites produced in liquid medium by 69 rhizobacteria strains for efficacy against second-stage juveniles (J_2) of *Meloidogyne exigua*, a species that is widespread in Brazilian coffee plantations, particularly in the States of Minas Gerais and São Paulo. Strains of the rhizobacteria tested (*Bacillus cereus, B. megaterium, B. pumilus, B. sphaericus, B. thuringiensis, Enterobacter asburiae, E. cloacae, E. intermedius, Kluyvera cryocrescens, Microbacterium liquefasciens, Paenibacillus macerans* and *Stenotrophomonas maltophilia*) are on deposit at the Department of Plant Pathology—Federal University of Lavras, State of Minas Gerais, Brazil. They were grown in tryptic soy broth (TSB, Merck) during ten days, at 25°C, under constant stirring (100 cpm), with no incidence of light. After bacterial cell removal by centrifugation (10,000*g*, 15 min), the supernatant liquids were used in the in vitro assay with *M. exigua*, performed as described by Amaral et al. (2003).

Among the 69 strains studied, 23 had no adverse effect on the juveniles. No correlation between

nematicidal activity and bacterial species or plants from which they were isolated could be observed. Mortality values as high as 100% were obtained with *B. megaterium* (strain 54-06), *B. pumilus* (strains 85-10 and 85-19), *B. thuringiensis* (strain 57-25), *E. asburiae* (strain 62-04), *E. cloacae* (strain 58-20) and *P. macerans* (strain 62-12). The highest value relative to *B. cereus* (94%) was determined for the strain 83-01.

The strains (eight) listed above, rated as the most effective in the initial test, were selected for a new, subsequent experiment, which dealt with: (i) a in vitro re-evaluation of the nematicidal activity of their metabolites; and (ii) evaluation of the nematicidal activity of the bacterial metabolites through a biotest with *M. exigua*-inoculated coffee plants under greenhouse conditions. However, due to technical difficulties in producing large amounts of metabolites of *B. pumilus* strains 85-10 and 85-19 for this second experiment, they were replaced with *B. pumilus* strains 58-15, 62-20, 83-14 and 84-20. For this test, the supernatant liquids of the selected (ten) bacterial strains (Table 1) were filtered through a 0.22 μm membrane. A mixture of soil, sand and cattle manure (1:2:1-v/v/v) was disinfested with methyl bromide (150 ml m^{-3}) and poured into 3 l pots. One six-month old coffee plant (*Coffea arabica* cv. Catuaí Amarelo) was planted per pot, inoculated with 2,000 *M. exigua* eggs (20 ml of an aqueous suspension) and treated with the rhizobacterial supernatants (20 ml). After 90 days in a greenhouse, above ground parts of the plants were cut off and the roots were gently washed with water to allow the number of galls to be estimated. Finally, nematode eggs were extracted from the roots (Boneti and Ferraz 1981) and their number estimated under the microscope. This experiment was arranged in a randomized block design, with six blocks, each one comprised of the (ten) bacterial strains plus two controls (inoculated plants irrigated with water or treated with 20 ml of a 500 μg ml^{-1} Aldicarb aqueous solution). Statistical calculations were done using SISVAR software (Ferreira 2000) and values of J_2 mortality (%) underwent variance analysis using ANOVA; means were analyzed according to the Scott and Knott test ($P \leq 0.05$).

All bacterial supernatant liquids retained their in vitro nematicidal activities (Table 1), but with the exception of *B. pumilus* (strains 58-15 and 84-20), the values of dead J_2 were lower than those recorded in the initial test. No correlation could be established between the in vitro effects and the number of root galls and nematode eggs per plant. Only for *B. pumilus* strain 83-18, which showed one of the highest values of dead J_2 in vitro, the number of root galls and nematode eggs per plant determined in the biotest were also very high; all the remaining strains caused a significant decrease in the number of root galls compared to water control. The best results were obtained with *B. megaterium*

Table 1 Effects of rhizobacterial metabolites on *Meloidogyne exigua* in vitro and in coffee plants

Bacteria	In vitro assay	Coffee plants assay	
	Dead J2[a] (%)[b]	Galls/plant[b]	Eggs/plant[b]
Bacillus cereus (83-01)	67.6 c	8 a	63 b
Bacillus megaterium (54-06)	55.6 b	8 a	40 a
Bacillus pumilus (58-15)	90.0 e	13 a	61 b
B. pumilus (62-20)	65.3 c	16 b	30 a
B. pumilus (83-18)	83.1 e	39 e	261 f
B. pumilus (84-20)	88.0 e	10 a	64 b
Bacillus thuringiensis (57-25)	88.3 e	21c	174 e
Enterobacter asburiae (62-04)	89.0 e	12 a	294 g
Enterobacter cloacae (58-20)	75.5 d	11 a	152 d
Paenibacillus macerans (62-12)	88.6 e	12 a	101 c
Water	2.6 a	29 d	156 d
Aldicarb	71.6 d	13 a	113 c

[a] J2: *Meloidogyne exigua* second-stage juveniles

[b] Means within columns followed by different letters are significantly different at $P \leq 0.05$ according to the Scott and Knott test

(54-06), which significantly reduced both the number of root galls and nematode eggs. No report dealing with the ability of this microrganism to produce nematicidal substances was found in the literature, but cells of this bacterium have already been used in formulations to control nematodes (Al-Rehiayani et al. 1999). Although *P. macerans* cells have also been used to control nematodes (Neipp and Becker 1999), it was not as effective as *B. megaterium* to reduce the number of nematode eggs. In view of these results, it is possible to conclude that rhizobacteria metabolites, especially those produced by a strain of *B. megaterium* isolated from coffee rhizosphere, can reduce *M. exigua* population in coffee plants. Therefore, it is strongly recommended that it should be included in further studies dealing with the assessment of potential agents for the biocontrol of this nematode and, eventually, of other species of *Meloidogyne* that attack coffee plants.

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