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Endophytic bacteria for biocontrol of coffee leaf rust (*Hemileia vastatrix*)

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Abstract: Suppression of plant diseases due to the action of endophytic microorganisms has been demonstrated in several pathosystems. Several mechanisms may control the suppression of plant pathogens, either directly by antibiosis and competition, or indirectly by induction of plant resistance response. The objective of this work was to select endophytic bacterial strains from coffee leaves (F), roots (R) and stems (G) with biocontrol potential against coffee leaf rust (*Hemileia vastatrix*). Two hundred fifteen endophytic bacterial strains were evaluated in coffee leaf discs. Bacterial suspensions were applied on leaf discs, 72 and 24 hours before, after and simultaneously with the pathogen. Nine bacterial strains (116G, 123G, 36F, 137G, 14F, 109G, 115G, 3F, and 119G) showed to be effective in reducing the rust development. These selected bacterial strains were evaluated in coffee seedlings (*Coffea arabica* 'Mundo Novo'). The bacterial suspensions were sprayed to the foliage 72 and 24 hours before, after, and simultaneously with the pathogen. The best control levels were obtained when the biocontrol agents were applied 72 hours before the pathogen. Four endophytic strains - 119G, 3F, 115G, and 109G - were effective in controlling coffee leaf rust (89, 84, 69 and 66%, respectively). The activity of enzymes (peroxidase, lipoxygenase, and phenylalanine ammonia-lyase) was assessed in relation to the control of *H. vastatrix* in leaf coffee seedlings seven days after the spray of four bacterial strains (119G, 3F, 115G, and 109G). It was observed that the inoculation of the strains 3F and 119G increased the peroxidase activity in leaves of coffee seedlings and significantly reduced the number of rust lesions per leaf. The other enzymes were not affected. The detection of peroxidase activity in leaves without the presence of the antagonists and pathogen proves the induction of systemic resistance, but probably there are other mechanisms of action. The isolates were identified based on cell membrane fatty acid contents, analyzed in a gas chromatograph, using microbial identification software (MIDI Sherlock TSBA Library version 5.0, Microbial ID, USA), as 3F=*Brevibacillus choschinensis*, 115G=*Microbacterium testaceum*, and 119G=*Cedecea davisae*.

Key words: biological control, *Coffea* spp.

Introduction

Beneficial endophytic microorganisms comprise especially fungi and bacteria that colonize internal plant tissues without causing visible damage to their hosts (Petrini, 1991). They are different from phytopathogenic microorganisms because they are not detrimental, do not cause diseases to plants, and are distinct from epiphytic microorganisms, which live on the surface of plant organs and tissues (Halmann et al., 1997). Endophytic bacteria are able to penetrate and become systemically disseminated in the host plant, actively colonizing the apoplast (Quadt-Hallmann et al., 1997b), conducting vessels (Hallmann et al., 1997), and occasionally the intracellular spaces (Quadt-Hallmann et al., 1997a). This colonization presents an ecological niche, similar to that occupied by plant pathogens, and these endophytic bacteria can therefore, act as biocontrol agents against pathogens (Hallmann et al.,

1997). The suppression of plant diseases due to the action of endophytic bacteria has been demonstrated in several patho-systems. Several mechanisms may control the suppression of plant pathogens, either directly by antibiosis and competition, or indirectly by induction of plant resistance response.

The coffee leaf rust caused by *Hemileia vastatrix* is the main disease in coffee, causing yield losses of 35 to 40%, on average. Control is basically achieved by fungicides. In 2000, in Brazil, the use of fungicides in coffee stood for 3,680 t of active ingredient (Campanhola & Bettiol, 2003). Therefore, alternatives control of coffee leaf rust must be sought. The objective of this work was to select endophytic bacteria strains from coffee leaves (F) and stems (G) with biocontrol potential against coffee leaf rust.

Material and methods

Bacterial isolates from leaves, and stems (Nunes, 2004), of *Coffea arabica* and *Coffea robusta* plants from São Paulo State, Brazil, were maintained in the culture collection of the Laboratory of Environmental Microbiology, Embrapa Environment. Two hundred fifteen isolates were evaluated in relation to their potential against coffee leaf rust.

Discs of young and completely developed leaves of *C. arabica* cv. Mundo Novo plants (susceptible to all *H. vastatrix* strains) were removed with a 2 cm diameter cork punch and placed into plastic boxes, abaxial surface facing up, over a layer of foam saturated with water. Bacterial suspensions were applied on leaf discs, 72 and 24 hours before, after and simultaneously with the same volume (25 µl) of *H. vastatrix* urediniospores suspension (1 mg ml^{-1}). After inoculation, boxes were covered with glass plates and incubated in the dark for 24 hours. Then, the boxes were maintained under 12-h photoperiod, 500-1000 lux, 22±2°C, and approximately 100% relative humidity. The experiment was set up in an completely randomized design (n=3), represented by nine leaf discs each. Severity of the disease was evaluated 30 days after inoculation, using a rating scale from 1 to 5, according to the percentage of leaf area with lesions (0 = 0%; 1 = 0-2.5%; 2 = 2.5-5%; 3 = 5-15%; 4 = 15-25%; and 5 ≥ 25% of leaf area with lesions).

Nine strains, 116G, 123G, 36F, 137G, 14F, 109G, 115G, 3F, and 119G, that showed to be effective in reducing the rust were then evaluated in coffee seedlings (*C. arabica* cv. Mundo Novo). The bacterial suspensions [A_{550}=0.1 (10^8 CFU ml^{-1})] were sprayed to the foliage 72 and 24 hours before, after, and simultaneously with the pathogen. After inoculation with the *H. vastatrix* urediniospores suspension (1 mg ml^{-1}), plants were incubated in the dark for 48 hours at 22±2°C at 100% relative humidity and then transferred to a greenhouse. Plants were irrigated daily and after 30 days the number of lesions per inoculated leaf was evaluated. Sterilized water was used as control. Trial was set up in a randomized blocks (n=10).

The activity of enzymes (peroxidase, lipoxygenase, and phenylalanine ammonia-lyase) was assessed in relation to the control of *H. vastatrix* in leaf coffee seedlings seven days after the spray of four bacterial strains (119G, 3F, 115G, and 109G).

The isolates were identified based on the cell membrane fatty acid contents (FAME), analyzed in a gas chromatograph, using microbial identification software (MIDI Sherlock TSBA Library version 5.0, Microbial ID, USA).

Results and discussion

Nine bacterial strains (116G, 123G, 36F, 137G, 14F, 109G, 115G, 3F, and 119G) out of 215 endophytic bacterial tested for their capacity of controlling the coffee leaf rust showed to be effective in reducing the disease in leaf disc assay.

In the test with coffee plants, the endophytic bacterial strains were not effective in controlling coffee leaf rust when applied after inoculation of the pathogen. The best control levels were obtained when the biocontrol agents were applied 72 hours before the pathogen. Four endophytic strains - 119G, 3F, 115G, and 109G - were effective in controlling the coffee leaf rust (89, 84, 69, and 66%, respectively). It was observed that the inoculation of the strains 3F and 119G increased the peroxidase activity in leaves of coffee seedlings and significantly reduced the number of rust lesions per leaf. The other enzymes were not affected (Table 1). The isolates were identified as 3F=*Brevibacillus choschinensis*, 115G=*Microbacterium testaceum*, and 119G=*Cedecea davisae*, based on cell membrane fatty acid contents.

The efficiency of certain endophytic bacterial strain in controlling coffee leaf rust can vary according to the time of biocontrol agent application. In general, the endophytes were more effective when applied 72 before inoculation of *H. vastatrix* urediniospores. The fact that the endophytic isolates showed activity when applied before the pathogen suggests that these isolates may act by antibiosis, lysis of pathogen structures, competition, or induction of systemic resistance in the host. The detection of peroxidase activity in leaves without the presence of the antagonists and pathogen proved the induction of systemic resistance, but probably there are other mechanisms of action involved (Table 1).

Even though a relatively small number of endophytic bacteria were tested, promising results were obtained regarding to the selection of coffee leaf rust biocontrol agents. Further field studies must be conducted in order to analyze the real potential of endophytic bacteria in field conditions. Studies are also needed to determine the action mechanisms of those bacteria, the population density of the applied endophytes, and the best form of introduction into the host.

Table 1. Effect of endophytic bacteria on lypoxygenase, phenylalanine ammonia-lyase, and peroxidase activity in coffee seedlings (*Coffea arabica* cv. Mundo Novo) and on disease

Strain	Lypoxygenase[1]	Phenylalanine ammonia-lyase[2]	Peroxidase[3]	Disease control (%)
3F	0.18 a	38 a	96 a	84
109G	0.15 a	29 a	89 a	66
115G	0.14 a	36 a	58.b	69
119G	0.13 a	35 a	54 b	89
Control	0.13 a	32 a	50 b	-

Means followed by the same letter are not significantly different (Tukey 5%). The percentage of disease control of the disease is relative to the control treatment obtained when the biocontrol agents were applied 72 hours before the pathogen.

[1] $\mu mol\ min^{-1}$ protein mg^{-1}.

[2] Δ absorbance unit min^{-1} protein mg^{-1}.

[3] nmol cinamic acid min^{-1} protein mg^{-1}.

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