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Biocontrol potential of endophytic strains of *Trichoderma asperellum* against *Fusarium oxysporum* f. sp. *cubense*, subtropical race 4

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RESUMEN

En este trabajo, se aislaron 10 cepas de *Trichoderma asperellum* endofíticas de iferentes genotipos de *Musa* spp. y se evaluó su potencial de biocontrol contra *Fusarium oxysporum* f. sp. *cubense* (Foc, raza 4 Subtropical), el agente causal de la marchitez por *Fusarium* del banano (MFB). Todas las cepas de *T. asperellum* mostraron potencial de biocontrol contra Foc. Las cepas YG01F, M001F y MC01F aisladas de Yamgambi No.2, *Musa acuminata* y *Musa acuminata* subsp. *malaccensis*, respectivamente, mostraron los mayores porcentajes de inhibición. Nuestros resultados sugieren una gran diversidad de *T. asperellum* endofíticos proceentes de raíces de *Musa* spp., con un gran potencial para el desarrollo de nuevos productos destinados a reducir el impacto de la MFB y mejorar la productividad en el banano.

Palabras clave: Banano, Marchitez por *Fusarium*, *Trichoderma*, biocontrol, Fitosanidad.

ABSTRACT

In this work 10 endophytic *Trichoderma asperellum* strains were isolated from different banana genotypes and evaluated for its biocontrol potential against *Fusarium oxysporum* f. sp. *cubense* (Foc, subtroopical race 4 -SR4) the causal agent of *Fusarium* wilt of banana (FWB). All the *T. asperellum* strains showed biocontrol potential against Foc. the strains YG01F, M001F, and MC01F from Yamgambi No.2, *Musa acuminata*, and *Musa acuminata* subsp. *malaccensis*, respectively, exhibited the highest percentages of inhibition. Our results suggest a high and multifunctional diversity of culturable endophytic *T. asperellum* from *Musa* spp. roots, with a strong potential for new product developments to reduce FWB impact and enhance productivity in banana.

Keywords: Banana, *Fusarium* wilt of banana, *Trichoderma*, biocontrol, phytosanity.

INTRODUCTION

Plants are populated with different sets of microorganisms with potential roles on host and ecosystem functions. Endophytic microorganisms colonize root tissues inter- and/or intracellularly producing a wide range of compounds useful for plants growth as well as for protection against biotic and abiotic stresses (Kavino and Manoranjitham, 2018).

Clonally propagated crops such as banana are frequently multiplied by using tissue culture techniques to get uniform and pathogen-free plants. This process, however, also eliminates the endophyte beneficial microbiota and might have plant fitness altered. *Trichoderma* spp. a multipurpose microorganism, utilizes direct antagonism and competition in the rhizosphere, colonizes plant roots (externally and/or as an endophyte), and has the capacity to promote plant growth and prime local and systemic defense responses against biotic stresses (Woo et al., 2022).

Fusarium wilt (FWB), caused by the soil-borne pathogen *Fusarium oxysporum* f. sp. *cubense* (Foc) is currently considered the major threat of the global banana production (Dita et al., 2018). The best practice to manage FWB is the use of resistant varieties. However, fully resistant cultivars to replace Cavendish or other susceptible commercial varieties are not yet available. Therefore, other management strategies such as the use of biological control agents (BCAs) are needed.

Trichoderma spp. is by far the most studied fungi for controlling FWB (Bubici et al., 2019). However, one of the drawbacks frequently raised about BCAs is the fitness of strains in field conditions and whether they can positively interact with host plants. A straightforward solution would be using BCA strains isolated from the target crop and environmental conditions. In this work, we screened different banana genotypes, isolated, identified and evaluated de biocontrol potential against Foc of 10 endophytic strains of *T. asperellum*.

MATERIAL AND METHODS

Isolation of endophytic *Trichoderma* spp. Samples from roots of 10 *Musa* spp. genotypes were processed using the protocol described by Pocasangre and Sikora (2000). Primary roots were split into two longitudinal sections and placed in a 2% sodium hypochlorite solution for three minutes, then washed with sterile tap water three times (3 min each) and split once into two longitudinal sections. Root sections were

placed on autoclaved paper towels to remove excess water and the outer layer of the root was peeled off with a scalpel. Internal roots tissues were cut into small pieces of approximately 1 to 1.5 cm length and placed on Petri´s dishes with Potato Dextrose Agar of 10% strength supplemented with 0.3 g streptomycin. The plates were incubated at 27 °C in the dark and after eight days colonies visually resembling *Trichoderma* spp. were sub-cultured to new plates for purification.

Identification of *Trichoderma* spp. DNA from colonies morphologically identified as *Trichoderma* spp. was extracted (Qiagen) and analyzed by sequencing the elongation factor (Tefa-1α) gene (O'Donnell et al., 1998) and the Internal Transcribed Spacer (ITS) region (White et al., 1990). PCR products were confirmed by electrophoresis on agarose gel of 0.8%. Sequencing was performed at MACROGEN using purified PCR products at a concentration of 50 ng ul-1. Sequencing products were compared to DNA databanks (NCBI) using the Blast (n) tool.

Antagonisms assays. Antagonisms assays were carried using a *Fusarium oxysporum* f. sp. *cubense* subtropical race 4 strain (VR039) isolated from Cavendish (AAA) banana located in the Ribeira Valle and previously characterized as VCG120. Screening for antagonistic activity against Foc was carried out usuing dual culture method described by Xianmei Yu et al. (2011). Sterile 90 mm Petri's dishes containing PDA were inoculated with each *T. asperellum* and cultivated next to the Foc strain. The mycelia plug (5 mm in diameter) taken from ten days old actively growing colonies of Foc was placed three days before the *Trichoderma* strains. Plates with pure cultures of either *Foc* or *Trichoderma* strains were used as controls. All plates were incubated under controlled conditions at 27 °C. Evaluations started at seven days after the assay setup, at which point the *Trichoerma* spp. control treatments covered the entire plate. The evaluation involved measuring the colony diameter and the antibiotic halo. At 21 days after the assay setup the occurrence or absence of mycoparasitism was evaluated using s scale of 0-3, where 3 represents the highest level of mycoparasitism (0 - no parasitism; 1: 1% -25%; 2: 26%-50%; 3: >50%). The percentage of inhibition of radial growth (PIRG) was measured by the formula: PIRG= (A-B/A) x 100, where A corresponds to the radial growth of the control pathogen plates and B the radial growth of the pathogen in coculture. The experimental design was completely randomized with three repetitions by treatments and control. The data underwent analysis of variance, and the means were compared (Tukey ≤ 0.05).

RESULTADS AND DISCUSSION

Trichoderma species were successfully isolated from all studied *Musa* spp. genotypes. In addition to *T. asperellum*, other species like *T. konigiopsis* and *T. spirale* were also identified (Data not shown). However, *T. asperellum* was the predominant species. For this study, one *T. asperellum* strain was selected per Musa genotype (Table 1). Further analysis are currently undergoing for the remaining isolates of *Trichoderma* spp.

Even when inoculated on plates three days after Foc, all *T. asperellum* isolates demonstrated the ability to inhibit the Foc (VR039). Although there was no statistical difference, variation was observed (Table 1, Figure 1). Notably, the strains YG01F, M001F, and MC01F from Yamgambi No.2, *Musa acuminata*, and *Musa acuminata* subsp. *malaccensis*, respectively, exhibited the highest percentages of inhibition (Table 1). All isolates demonstrated the capability to mycoparasitize Foc, but variations were observed. Two isolates exhibited grade 1 mycoparasitism (up to 25% pathogen colonization), three isolates presented grade 2 mycoparasitism (26% to 50% pathogen colonization), while the remaining five isolates demonstrated grade 3 mycoparasitism (more than 50% pathogen colonization).

Table 1. List of *Trichoderma asperellum* strains and their behavior against *Fusarium oxysporum* f. sp *cubense*.

Code	Genotype	Genome	Growth inhibition of Foc (%)	Mycoparasitism (Scale 0-3)
B001F	*Musa balbisiana*	BB	30	1
M001F	*Musa acuminata*	AA	38	2
MC01F	*Musa malaccensis*	AA	37	3
OR002F	*Musa ornata royal*	*	33	3
PB002F	Pisang Bungai	AA	35	2
PCV01F	Pacovan	AAB	36	2
PJB01F	Pisang Jaru Buaya	AA	36	3
PS029F	Pisang Seribu	AAB	30	1
SB01F	Saba	ABB	35	3
YG01F	Yamgambi Km2	AAB	43	3

*Wild specie with unknown genome

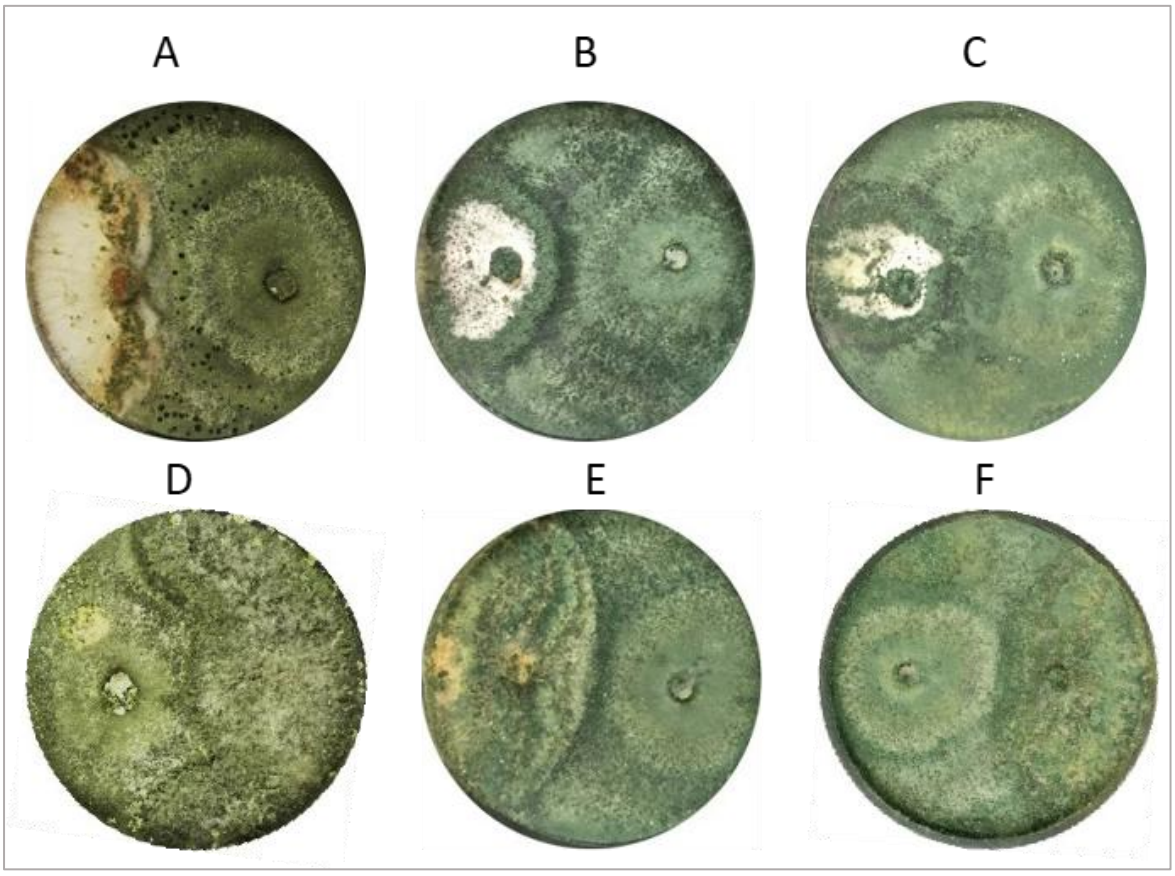

Figure 1. General overview of growth inhibition and mycoparasitism caused by *Trichoderma asperellum* strains on *Fusarium oxysporum* f. sp. *cubense* at 21 days after co-inoculation. **A**: B001F. **B**: PCV01F. **C**: M001F. **D:** MC01F. **E**: PJB01F. F: YG001F.

CONCLUSION

All *Trichoderma* strains assessed showed antagonism and mycoparasitism capacity against Foc by restricting it radial growth expansion of the pathogen and colonizing the surface of mycelial Foc and subsequently sporulation on it. Considering both PIGR and mycoparasitism levels, isolates YG01F and M001F could be considered, in a preliminary assessment, as more promising BCAs. Further studies at greenhouse and field levels are undergoing to validate the biocontrol potential against FWB of these *T. asperellum* strains.

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