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Antagonistic potential of endophytic bacteria from tomato seedlings

Abstract – The objective of this work was to evaluate the antagonistic potential of endophytic bacteria isolated from 'Cereja Vermelho', 'Rasteiro Rio Grande', and 'Chapolin' tomato seedlings against the ALT23 *Alternaria* sp. fungus. Of the 23 endophytic isolates obtained, 12 were selected due to their greater effect on the mycelial growth of ALT23. Antagonism was tested in a completely randomized design, with 13 treatments (ALT23 and 12 isolates), with four replicates. The selected endophytic bacteria inhibited from 29.8 to 64.2% of the mycelial diameter and from 33.7 to 72.4% of the growth rate of ALT23.

Index terms: *Bacillus*, *Burkholderia*, *Solanum lycopersicum*, *Paenibacillus*.

Potencial antagônico de bactérias endofíticas de plântulas de tomate

Resumo – O objetivo deste trabalho foi avaliar o potencial antagônico de bactérias endofíticas isoladas de mudas de tomate 'Cereja Vermelho', 'Rasteiro Rio Grande' e 'Chapolin' contra o fungo *Alternaria* sp. ALT23. Dos 23 isolados endofíticos obtidos, 12 foram selecionados por terem apresentado maior efeito sobre o crescimento micelial do ALT23. O antagonismo foi testado em delineamento inteiramente casualizado, com 13 tratamentos (ALT23 e 12 isolados), com quatro repetições. As bactérias endofíticas selecionadas inibiram de 29,8 a 64,2% o diâmetro micelial e de 33,7 a 72,4% a velocidade de crescimento do ALT23.

Termos para indexação: *Bacillus*, *Burkholderia*, *Solanum lycopersicum*, *Paenibacillus*.

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Tomato (*Solanum lycopersicum* L.), a vegetable of global economic importance, is affected by the *Alternaria* spp. phytopathogenic fungus, which causes black spots on its fruits, leaves, and stems, as well as seed rot, seedling damping off, and a reduced fruit quality (Töfoli & Domingues, 2018). In an ecological and sustainable way, using endophytic microorganisms can promote the biological control of the phytopathogen without contaminating the environment and leaving toxic residues in the food, also stimulating plant growth and reducing the use of pesticides in agricultural fields (Morales-Cedeño et al., 2021; Malik et al., 2024).

Endophytes, such as those of the genera *Bacillus* and *Burkholderia*, can release volatile secondary metabolites and/or lipopeptides that have shown antagonistic potential against several phytopathogens (Morales-

Cedeño et al., 2021; Malik et al., 2024). The *Bacillus subtilis* bacteria isolated from sugarcane (*Saccharum officinarum* L.) leaves, for example, has shown antifungal action against *Fusarium* (Hazarika et al., 2019). In tomato plants, the *Burkholderia contaminans* and *Bacillus velezensis* endophytic bacteria reduced the incidence of the *Sclerotium rolfsii* and *Botrytis cinerea* phytopathogenic fungi (Hari et al., 2023; Feng et al., 2024). However, no reports were found on the use of endophytic bacteria from Brazilian tomato cultivars, such as Cereja Vermelho, which may be better adapted to the host plant and more efficient in controlling phytopathogens compared with microorganisms obtained from other types of plants.

The objective of this work was to evaluate the antagonistic potential of endophytic bacteria isolated from 'Cereja Vermelho', 'Rasteiro Rio Grande', and 'Chapolin' tomato seedlings against the ALT23 *Alternaria* sp. fungus.

The bacterial isolates were obtained from seedlings of three tomato plants from the following cultivars: Cereja Vermelho, Rasteiro Rio Grande, and Chapolin, whose seeds were produced by ISLA Sementes Ltda. (Porto Alegre, RS, Brazil). The isolate of the phytopathogenic fungus *Alternaria* sp. used was ALT23 from the Agricultural Microbiology Laboratory of the Department of Agronomic Engineering of Universidade Federal de Sergipe, located in the municipality of São Cristóvão, in the state of Sergipe, Brazil.

The antagonistic potential of the selected endophytic bacteria was evaluated in a bioassay performed in a completely randomized experimental design with 13 treatments (ALT23 and 12 bacterial isolates selected), with four replicates.

Twenty-five seeds per cultivar were superficially disinfected, with two replicates, according to Alfenas & Mafia (2007). Subsequently, the seeds were placed to germinate in a plastic box with three sheets of autoclaved filter paper, moistened with autoclaved distilled water, and incubated at 28±1°C over a photoperiod of 8 hours of light during five days. On the second day of incubation, 5.0 mL autoclaved distilled water were added. After five days, the seedlings with the greatest vegetative development were selected, and, from these, four fragments of up to 0.5 cm were removed from the radicle and/or leaflets and transferred to Petri dishes with potato-dextrose-agar (PDA) medium (39 g L^{-1} commercial PDA), with three replicates per cultivar. Incubation was carried out at 28±1°C with a photoperiod of 8 hours of light for five days. The number of endophytic bacteria per cultivar was determined after the serial dilution of bacterial colonies up to 10^{-3} according to Lima et al. (2024). The bacterial colonies obtained were quantified and identified using the subsequent acronyms referring to the three used cultivars, followed by a numerical identification sequence: CB, Cereja Vermelho; RB, Rasteiro Rio Grande; and HB, Chapolin. The letters A (yellow colonies) or T (light to clear cream-colored colonies) were added to differentiate the isolates obtained from the same seedling.

To test the antagonistic potential of the endophytic bacteria isolated from the tomato seedlings, a 5.0 mm disc of the culture medium colonized by the ALT23 fungus was transferred to the center of a Petri dish containing a commercial PDA culture medium. Four autoclaved filter paper discs, moistened in autoclaved distilled water containing a bacterial isolate culture, were placed around the disc and incubated for five days, at 28±1°C, under a photoperiod of 8 hours. The inhibition of the mycelial growth of the ALT23 phytopathogenic fungus was visually classified as: moderate, <40%; strong, >40%; and very strong, >70% (Figure 1).

A total of 23 endophytic bacterial isolates were obtained, as follows: 6 from 'Vermelho Cereja' (CB1 to CB6), 11 from 'Rasteiro Rio Grande' (RB9A to RB16), and 6 from 'Chapolin' (HB17 to HB23). From these 23 isolates, 12 were selected due to their greater effect on the mycelial growth of ALT23, which underwent strong changes with 11 isolates (CB2, CB3, CB5, CB6, RB9A, RB12, RB13, RB14, RB15T, HB19A, and HB22A) and a very strong change with one isolate (RB10T). The obtained endophytic bacteria species were identified according to the method described by Lima et al. (2024).

The antagonism of the 12 selected endophytic bacteria was evaluated using the same method described previously for the selection of isolates from the tomato seedlings, but with 2.5 cm between the mycelial disc of the fungi and the bacterial inoculums, as well as incubation at ambient temperature for five days. The mycelial diameter in centimeters, growth rate in centimeters per day, percentage of mycelial diameter, and percentage of the growth rate of ALT23

were calculated according to Lima et al. (2024). The odor of the cultivation medium was classified as mild, strong, or absent.

The data were subjected to the analysis of variance, and, when a significant difference was observed, Tukey's test was applied at 5% probability using the SISVAR, version 5.8, software (Ferreira, 2011).

The isolates from 'Cereja Vermelho', 'Rasteiro Rio Grande', and 'Chapolin' tomato seedlings obtained in the present study showed a great genetic similarity with some species of the genus *Bacillus* (Table 1). All 12 selected endophytic bacterial isolates significantly reduced the mycelial diameter and growth rate of the ALT23 *Alternaria* sp. fungus when compared with the control (Table 2).

Among the selected isolates of *Bacillus* spp., RB10T inhibited the mycelial diameter and growth rate of ALT23 in 64.21 and 72.44%, respectively, representing values higher than that of 58% reported by Shuang et al. (2022) when using the K-9 *Bacillus* sp. strain against *Alternaria* sp. However, RB12 inhibited only 43.75% of the mycelial diameter and 49.36% of the growth rate of ALT23, as observed when using this species of bacteria obtained from cherry plum (*Prunus cerasifera* Ehrh.) against *Fusarium* sp. (Pei et al., 2023).

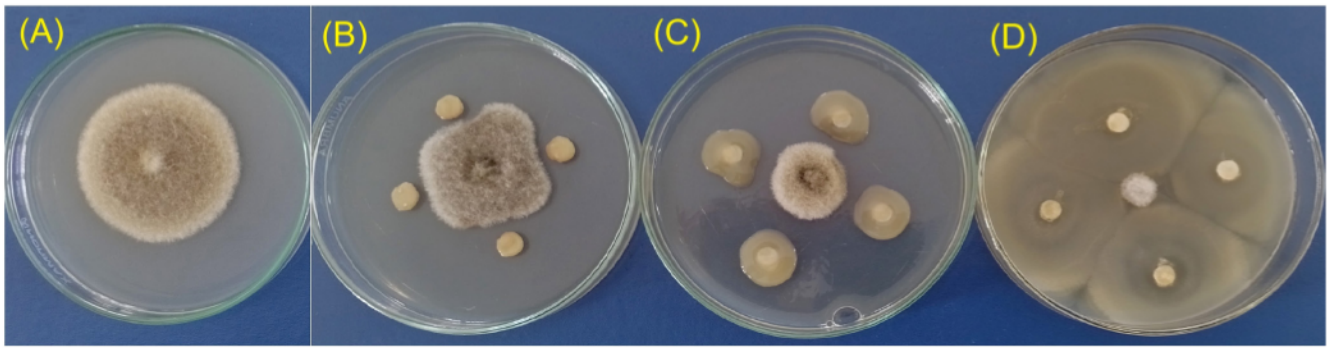

Figure 1. Mycelial growth of the ALT23 *Alternaria* sp. fungus in typical form (A) and in the presence of endophytic bacteria from tomato (*Solanum lycopersicum*) seedlings, showing moderate (B), strong (C), and very strong (D) alterations after five days.

Table 1. Identification of the endophytic bacteria isolated from seedlings of different tomato (*Solanum lycopersicum*) cultivars and evaluated as to their capacity to inhibit the mycelial growth of the ALT23 *Alternaria* sp. phytopathogenic fungus.

Tomato cultivar	Bacterial isolate	Bacterial species	Similarity (%)	GenBank accession number
Cereja Vermelho	CB2	MS 2379 *Paenibacillus ottowii*	99.82	PP157597
	CB3	KCTC 13613 *Bacillus siamensis*	99.93	PP157598
	CB5	MS 2379 *Paenibacillus ottowii*	99.56	PP157599
	CB6	MS 2379 *Paenibacillus ottowii*	99.32	PP157600
Rasteiro Rio Grande	RB9A	MS 2379 *Paenibacillus ottowii*	99.55	PP157603
	RB10T	CR-502 *Bacillus velezensis*	100.00	PP157604
	RB12	NCIB 3610 *Bacillus subtilis*	100.00	PP157605
	RB13	MS 2379 *Paenibacillus ottowii*	99.57	PP157606
	RB14	MS 2379 *Paenibacillus ottowii*	99.47	PP157607
	RB15T	CR-502 *Bacillus velezensis*	99.85	PP157608
Chapolin	HB19A	LMG 28158 *Burkholderia territorii*	99.79	PP157601
	HB22A	LMG 28158 *Burkholderia territorii*	99.79	PP157602

Isolates CB2, CB5, CB6, RB9A, RB13, and RB14, classified as the closest species to *Paenibacillus ottowii*, reduced the mycelial diameter and growth rate of ALT23 by 43.18 to 49.15% and by 48.72 to 55.45%, respectively. HB19A and HB22A from *Burkholderia territorii* showed the lowest percentages of mycelial diameter and growth inhibition, which varied from 29.83 to 30.68% and from 33.66 to 34.62% (Table 2), respectively, representing values similar to that of 32% obtained for the *Burkholderia* sp. isolate against *Giberella zeae* (Brito et al., 2018).

The alteration in the mycelial growth of ALT23 in the presence of endophytic isolates was: moderate, for HB19A and HB22; strong, for CB2, CB3, CB5, CB6, RB13, RB14, and RB15T; and very strong, for RB10T. According to Malik et al. (2024), antagonistic bacteria release lipopeptides capable of causing damage to hyphae and altering the mycelial growth of phytopathogens. Other authors added that *Bacillus* sp., *Burkholderia* sp., and *Paenibacillus* sp. inhibit mycelial growth and release volatile antimicrobial compounds (Morales-Cedeño et al., 2021; Hari et al., 2023), which may have contributed to the presence of a mild odor in RB15T, in addition to strong odors in RB10T, RB12, CB2, CB5, CB6, and RB9A. However, the RB13, RB14, HB19A, and HB22A endophytes did not exhibit a characteristic odor, which may have influenced their low rate of inhibition of the mycelial growth of ALT23.

The endophytic bacteria isolated from 'Cereja Vermelho', 'Rasteiro Rio Grande', and 'Chapolin' tomato seedlings have the potential to control the ALT23 phytopathogenic fungus. However, it is necessary to evaluate both the synergism of these microorganisms under field conditions and their antagonism against other phytopathogens in future works.

Table 2. Mycelial diameter (MD), inhibition of the mycelial diameter (I-MD), growth rate (GR), and inhibition of the growth rate (I-GR) of the ALT23 *Alternaria* sp. fungus in the presence of endophytic bacteria isolated from the Cereja Vermelho (CB), Rasteiro Rio Grande (RB), and Chapolin (HB) tomato (*Solanum lycopersicum*) cultivars after five days of cultivation[(1)].

Treatment	MD (cm)	I-MD (%)	GR (cm per day)	I-GR (%)
Control	4.40a	-	0.78a	-
CB2	2.34bcde	46.88	0.37bcde	52.88
CB3	2.06de	53.13	0.31de	59.94
CB5	2.36bcde	46.31	0.37bcde	52.24
CB6	2.24cde	49.15	0.35cde	55.45
RB9A	2.35bcde	46.59	0.37bcde	52.57
RB10T	1.58e	64.21	0.22e	72.44
RB12	2.48bcd	43.75	0.40bcd	49.36
RB13	2.40bcd	46.46	0.38bcd	51.28
RB14	2.50bcd	43.18	0.40bcd	48.72
RB15T	2.41bcd	45.17	0.38bcd	50.96
HB19A	3.05bc	30.68	0.51bc	34.62
HB22A	3.09b	29.83	0.52b	33.66
CV (%)	12.9		16.0	

[(1)]Means followed by equal letters, in the column, do not differ from each other by Tukey's test, at 5% probability.

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