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Rhizobacteria inhibit the growth of *Fusarium oxysporum* f. sp. *phaseoli* in vitro

Tayla Évellin de Oliveira*, Ana Carolina Rodrigues Alves, Maria de Lourdes Resende, Adauton Vilela de Rezende and Ligiane Aparecida Florentino

Universidade Professor Edson Antônio Velano, Rodovia MG-179, 37132-440, Alfenas, Minas Gerais, Brazil. *Author for correspondence. E-mail: tayla.oliveira@unifenas.br

ABSTRACT. Wilt caused by the fungus *Fusarium oxysporum* f. sp. *phaseoli* is one of the major diseases affecting common bean cultivation. Due to the significant damage caused by this pathogen, rhizobacteria have emerged as a promising alternative for its biological control. This study aimed to evaluate the potential of rhizobacteria to inhibit *F. oxysporum* f. sp. *phaseoli* in vitro. The antagonistic effect of 20 bacterial strains was assessed using four distinct methods: the circle technique, dual culture, central streak pairing, and fungal culture over antagonist culture. Strains that exhibited antagonistic activity in at least three of the methods were grouped into a bacterial consortium, which was also tested for inhibitory potential. A completely randomized design with three replicates was used. Evaluations were conducted on the tenth day, including measurements of fungal colony diameter and calculation of the percentage of growth inhibition (PGI). The bacterial strains, both individually and in combination, were effective in inhibiting the mycelial growth of the fungus, particularly in the circle and fungal-over-antagonist culture techniques, highlighting their potential as viable agents for the biological control of *F. oxysporum* f. sp. *phaseoli*.

Keywords: alternative control; antagonistic activity; *Phaseolus vulgaris* L.; wilt.

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Introduction

Common bean (*Phaseolus vulgaris* L.) plays a fundamental role in agriculture and is considered a staple food in the diet of millions of people due to its accessibility as a source of plant-based protein and fiber (Uebersax et al., 2023). However, its production can be affected by several factors, including the incidence of pests and diseases. Among them, Fusarium wilt stands out, caused by the fungus *Fusarium oxysporum* f. sp. *phaseoli* (Fop), whose remarkable ability to survive in the soil, even in the absence of a living host, contributes to its persistence and wide distribution. This characteristic makes the pathogen one of the main causes of yield reduction in common bean crops (Benchimol-Reis et al., 2023; Ngoya et al., 2024).

The use of resistant cultivars is the primary strategy for disease management (Leitão et al., 2020). Nevertheless, it is important to note that a single cultivar may not be resistant to all pathogen races, especially due to the high pathogenic variability observed in Fop. Additionally, many farmers still choose susceptible cultivars because of their desirable agronomic traits or due to market demands. This scenario highlights the need for complementary management strategies aimed at reducing disease incidence and mitigating its impact on crop production (Borba et al., 2017; Elhelaly & Ammar, 2022).

Given this context, chemical control through seed treatment with fungicides remains widely used. However, this practice may lead to undesirable consequences, such as the emergence of resistant isolates and concerns related to food safety and environmental impact. As an alternative, there is growing interest in the use of biological control agents, which are characterized by low toxicity, relatively low cost, and the ability to act specifically against the etiological agent (Oliveira et al., 2020; Mabaso et al., 2025).

One possible strategy for controlling Fop involves the use of soil bacteria known as rhizobacteria, named for their ability to colonize plant roots. In addition to promoting plant growth, these bacteria can inhibit pathogen development through direct antagonism or by inducing plant resistance (Vejan et al., 2016; Wang et al., 2021). Therefore, the use of rhizobacteria represents a sustainable and promising approach for managing Fusarium wilt. Based on this context, the objective of this study was to evaluate the potential of rhizobacteria in inhibiting *F. oxysporum* f. sp. *phaseoli*.

Material and methods

Identification and acquisition of bacterial strains and the phytopathogen *Fusarium oxysporum* f. sp. *phaseoli*

Twenty bacterial strains were used, originating from the collection of the Soil Microbiology Laboratory (LMS) at *Universidade Professor Edson Antônio Velano* (UNIFENAS), previously isolated by Florentino et al. (2014). Table 1 presents the identification and morphological characteristics of the strains grown on 79 medium (Fred & Waksman, 1928).

Table 1. Identification and morphological characterization of bacterial strains grown in culture medium 79 using bromothymol blue indicator isolated from root nodules of *Phaseolus vulgaris* and *Gliricidia sepium*.

Identification of strains	Morphological characteristics in medium 79		
	pH	Production of exopolysaccharides	Color
Strains isolated from *Phaseolus vulgaris*			
UNIFENAS 02-10	Acid	High	Cream
UNIFENAS 02-11	Acid	High	Yellow
UNIFENAS 02-12	Acid	High	Yellow
Strains isolated from *Gliricidia sepium*			
UNIFENAS 03-10	Acid	Low	Yellow
UNIFENAS 03-11	Acid	Low	Yellow
UNIFENAS 03-12	Acid/ Alkaline	Low	Yellow
UNIFENAS 03-14	Alkaline	Low	Cream
UNIFENAS 03-15	Acid/ Alkaline	Medium	Yellow
UNIFENAS 03-16	Acid	Low	Yellow
UNIFENAS 03-17	Acid	Medium	Yellow
UNIFENAS 03-23	Alkaline	Low	Cream
UNIFENAS 03-24	Alkaline	Low	Cream
UNIFENAS 03-25	Alkaline	Low	White
UNIFENAS 03-27	Neutral	Medium	Cream
UNIFENAS 03-28	Neutral	Medium	Cream
UNIFENAS 03-29	Neutral	Medium	Cream
UNIFENAS 03-31	Acid	Medium	Cream
UNIFENAS 03-33	Acid/ Alkaline	Medium	Yellow
UNIFENAS 03-35	Acid	Medium	Yellow
UNIFENAS 03-36	Neutral	Low	Yellow

All strains presented colonies with regular shape, production of exopolysaccharides with a gummy consistency, and rapid growth on culture medium. The *Rhizobium tropici* strain (CIAT 899) was also included in the tests.

The isolate of *F. oxysporum* f. sp. *phaseoli* was provided by the *Universidade Federal de Lavras* (UFLA) and was sub cultured onto Petri dishes containing autoclaved PDA medium (potato dextrose agar). The plates were incubated at 28°C under a 12-hour photoperiod and maintained in a BOD-type incubator for seven days to promote fungal multiplication.

Evaluation of enzymatic activity

The production of hydrolytic enzymes by bacterial strains was assessed through tests for amylolytic, lipolytic, and chitinolytic activities.

The amylolytic activity test was conducted according to Andualem and Gessesse (2013), using Petri dishes containing starch agar (1% soluble starch, 0.5% peptone, 1.5% yeast extract, and 1.5% agar). The strains were incubated at 28°C for 96 hours. Subsequently, 2 mL of Lugol iodine solution (1% iodine [I_2] and 2% potassium iodide [KI]) were added to the media. The presence of a clear halo around the colonies indicated amylase production.

Lipolytic activity was evaluated following the method of Sierra (1957), using a medium composed of 10 g peptone, 5 g NaCl, 0.1 g $CaCl_2$, 14 g agar, and 10 mL Tween 80. After incubation under the same conditions, the presence of a clear halo indicated a positive result.

For chitinase screening, a medium containing colloidal chitin as the sole carbon and energy source was used, following Kamil et al. (2007). The medium composition included 6 g disodium phosphate (Na_2HPO_4), 3 g dipotassium phosphate (K_2HPO_4), 1 g ammonium chloride (NH_4Cl), 0.5 g NaCl, 0.05 g yeast extract, 15 g agar, and 10 g chitin. Plates were incubated at 28°C for seven days, and the formation of a clear halo indicated enzyme production.

All tests were performed in triplicate and qualitatively evaluated, classifying the strains as positive or negative for each enzyme.

Evaluation of antagonistic activity

To perform the antagonism tests, bacterial strains were cultured in 79 medium at 28°C for 48 hours to allow the development of isolated colonies and confirm purity. The fungus was transferred to PDA medium and incubated for ten days at 28 ± 0.5°C.

Subsequently, four methods were applied. The first method consisted of the circle technique, in which a 5.0 mm diameter fungal mycelium disc was placed at the center of a Petri dish containing PDA medium, followed by bacterial inoculation around the disc (Mariano, 1993). The second method employed the paired culture technique, where the fungal disc was inoculated 1.5 cm from the plate edge and the bacterial strain at the opposite edge, also 1.5 cm from the border (Melo & Valarini, 1995). In the third method, the pairing technique with a streak in the center of the plate was used, in which bacteria were inoculated by streaking near the fungal disc (Dennis & Webster, 1971). Finally, the fourth method consisted of the fungal culture over antagonist culture technique, adapted from Braga Júnior et al. (2017), where bacterial strains were inoculated in the center of the plate and the fungal disc was subsequently placed over the bacterial culture.

For the control, only the fungal pathogen disc cultivated on PDA medium was used in all methods. All assays were performed in triplicate. After inoculation, the plates were incubated at 28 ± 0.5°C. Evaluations were conducted on the tenth day, with measurements of fungal colony diameters taken in two diametrically opposite directions using a caliper, and the average diameter was calculated for each colony.

The averages were used to calculate the percentage of growth inhibition (PGI), determined according to the formula of Menten et al. (1976), where:

$$\text{PGI} = \frac{\text{Control growth} - \text{Treatment growth}}{\text{Control growth}} \times 100$$

A completely randomized design was adopted in a 22 (21 bacterial strains + one control) x 4 (methods) factorial scheme with three replicates per treatment, with each Petri dish constituting an experimental unit. The data was subjected to analysis of variance and the means were compared using the Scott-Knott test at 5% significance using the R statistical software (R Core Team, 2023).

Bacterial mixtures

Aiming to use the antagonistic strains in combinations, those that showed the lowest average colony diameters in at least three of the tested methods were selected. These strains were then used to compose a bacterial mix. Table 2 presents the identification and the selected strains.

Table 2. Identification and combination of the bacterial strains selected to make up the bacterial mix.

Identification	Strains
Mix 1	UNIFENAS 03-14 + 03-23 + 03-27
Mix 2	UNIFENAS 03-14 + 03-23 + 03-33
Mix 3	UNIFENAS 03-14 + 03-23 + 03-36
Mix 4	UNIFENAS 03-14 + 03-23 + CIAT 899
Mix 5	UNIFENAS 03-14 + 03-27 + 03-33
Mix 6	UNIFENAS 03-14 + 03-27 + 03-36
Mix 7	UNIFENAS 03-14 + 03-27 + CIAT 899
Mix 8	UNIFENAS 03-14 + 03-33 + 03-36
Mix 9	UNIFENAS 03-14 + 03-33 + CIAT 899
Mix 10	UNIFENAS 03-14 + 03-36 + CIAT 899
Mix 11	UNIFENAS 03-23 + 03-27 + 03-33
Mix 12	UNIFENAS 03-23 + 03-27 + 03-36
Mix 13	UNIFENAS 03-23 + 03-27 + CIAT 899
Mix 14	UNIFENAS 03-23 + 03-33 + 03-36
Mix 15	UNIFENAS 03-23 + 03-33 + CIAT 899
Mix 16	UNIFENAS 03-23 + 03-36 + CIAT 899
Mix 17	UNIFENAS 03-27 + 03-33 + 03-36
Mix 18	UNIFENAS 03-23 + 03-33 + CIAT 899
Mix 19	UNIFENAS 03-23 + 03-36 + CIAT 899
Mix 20	UNIFENAS 03-33 + 03-36 + CIAT 899

To ensure that the selected strains could be combined without antagonism, a compatibility screening was performed using the cross-streak method before formulating the mix. In this assay, one strain was streaked longitudinally in a 7 cm line with a width of 0.5 cm, while the second strain was applied perpendicularly, crossing the first, maintaining a distance of 1 cm between the lines of the test pathogen. The plates were incubated at 28°C for 48 hours, and the observed growth allowed the identification of compatible strains for the mix formulation (Lertcanawanichakul & Sawangnop, 2008).

Only after confirming compatibility were the strains transferred to a single flask containing liquid culture medium 79 and incubated at 28°C for 48 hours to formulate the mix. Subsequently, 8 µL aliquots of the inoculant were pipetted onto Petri dishes containing medium 79, incubated for 24 hours, and then replica plated using a platinum loop.

The bacterial mixes were submitted to the four aforementioned methods to evaluate their antagonistic activity against the fungus, following the same conditions and procedures previously described.

A completely randomized design was adopted in a 21 (20 bacterial mixes + one control) x 4 (methods) factorial scheme with three replicates per treatment, with each Petri dish constituting an experimental unit. The data was subjected to analysis of variance and the means were compared using the Scott-Knott test at 5% significance using the R statistical software (R Core Team, 2023).

Results and discussion

The enzymatic activity of the bacterial strains regarding their ability to produce amylase, lipase, and chitinase is presented in (Table 3). It was observed that all strains were capable of synthesizing at least one of the tested enzymes, with the production of amylase, lipase, and chitinase observed in 30%, 85%, and 40% of the strains, respectively.

Table 3. Enzymatic activity of bacterial strains with antagonistic potential.

Strains	Amylolytic activity	Lipolytic activity	Chitinolytic activity
UNIFENAS 02-10	+	+	
UNIFENAS 02-11	+		
UNIFENAS 02-12	+		
UNIFENAS 03-10		+	+
UNIFENAS 03-11		+	+
UNIFENAS 03-12		+	+
UNIFENAS 03-14		+	+
UNIFENAS 03-15		+	
UNIFENAS 03-16	+		
UNIFENAS 03-17		+	
UNIFENAS 03-23		+	+
UNIFENAS 03-24		+	+
UNIFENAS 03-25		+	
UNIFENAS 03-27	+	+	
UNIFENAS 03-28		+	+
UNIFENAS 03-29	+	+	
UNIFENAS 03-31		+	
UNIFENAS 03-33		+	+
UNIFENAS 03-35		+	
UNIFENAS 03-36		+	

(+) Formation of a halo around the colony

The literature reports that antagonistic microorganisms can act in different ways to inhibit the activity of pathogens (Vejan et al., 2016; Tariq et al., 2020; Wang et al., 2021; Haq et al., 2024). One such approach involves the degradation of cell wall components through the action of hydrolytic enzymes (Riseh et al., 2024). This was documented by Basha and Ulaganathan (2002) in their investigation of the in vitro antagonistic activity of *Bacillus* sp. BC121 against *Curvularia lunata*. The strain demonstrated remarkable antagonistic activity against the pathogen, showing evidence of fungal cell wall degradation through scanning electron microscopy, as well as confirming its ability to secrete chitinase. These findings highlight the importance of understanding the enzymatic activity of these biocontrol agents.

In the experiment that evaluated the antagonistic activity of the bacterial strains against the fungus *F. oxysporum* f. sp. *phaseoli*, it was found that the strains exhibited an inhibitory effect on the mycelial growth

of the pathogen when subjected to laboratory tests using the four inhibition methods. The results related to the diameter of the fungal colonies and the PGI are presented in (Table 4).

Table 4. Inhibition of mycelial growth of *Fusarium oxysporum* f. sp. *phaseoli* by bacterial strains from root nodules of *Phaseolus vulgaris* and *Gliricidia sepium*.

Treatments	Methods 1		Methods 2		Methods 3		Methods 4	
	Ø (cm)	PGI (%)	Ø (cm)	PGI (%)	Ø (cm)	PGI (%)	Ø (cm)	PGI (%)
UNIFENAS 02-10	0 Aa	100	2.36 Bc	52.36	1.33 Bb	65.64	1.30 Ab	84.70
UNIFENAS 02-11	2.20 Cb	74.27	2.40 Bb	51.77	0.76 Aa	81.22	4.86 Cc	42.74
UNIFENAS 02-12	1.23 Ba	85.63	4.93 Db	0.65	2.00 Ca	49.54	1.56 Ba	81.57
UNIFENAS 03-10	0.40 Aa	95.34	2.80 Bc	43.65	1.43 Bb	62.46	0.73 Aa	91.37
UNIFENAS 03-11	0.60 Aa	93.05	1.90 Bb	61.89	1.60 Bb	59.23	0.66 Aa	92.16
UNIFENAS 03-12	0.53 Aa	93.77	3.20 Cb	35.49	1.53 Ba	61.01	0.90 Aa	89.41
UNIFENAS 03-14	0.60 Aa	93.00	0.93 Aa	81.16	0.70 Aa	80.60	0.96 Aa	88.63
UNIFENAS 03-15	5.53 Ec	35.35	4.46 Db	9.91	1.80 Ba	54.01	8.50 Dd	0.00
UNIFENAS 03-16	0.86 Aa	89.88	2.66 Bb	46.35	2.03 Cb	48.13	2.23 Bb	73.72
UNIFENAS 03-17	1.26 Ba	85.23	1.30 Aa	73.65	2.00 Cb	49.28	2.36 Bb	72.15
UNIFENAS 03-23	0.50 Aa	94.16	2.06 Bb	58.33	0.50 Aa	87.27	0.93 Aa	89.02
UNIFENAS 03-24	0.66 Aa	92.18	3.23 Cb	34.84	2.60 Cb	37.04	0.76 Aa	90.98
UNIFENAS 03-25	0.10 Aa	98.82	2.16 Bc	56.45	1.33 Bb	66.11	0.76 Aa	90.98
UNIFENAS 03-27	0.40 Aa	95.32	0.93 Aa	81.29	1.40 Ba	65.77	0.90 Aa	89.41
UNIFENAS 03-28	0.86 Aa	89.91	2.56 Bb	48.34	2.16 Cb	47.69	0.96 Aa	88.62
UNIFENAS 03-29	0.33 Aa	96.08	1.90 Bb	61.65	1.20 Bb	69.93	1.70 Bb	80.00
UNIFENAS 03-31	0.20 Aa	97.67	3.66 Cc	26.00	1.46 Bb	64.17	0.96 Ab	88.62
UNIFENAS 03-33	0.30 Aa	96.51	1.96 Bb	60.37	0.90 Aa	77.71	0.73 Aa	91.37
UNIFENAS 03-35	4.03 Dc	52.77	2.26 Bb	54.32	0.93 Aa	74.73	5.46 Cd	35.68
UNIFENAS 03-36	0.86 Aa	89.91	1.26 Aa	74.28	0.90 Aa	77.71	1.83 Ba	78.43
CIAT 899	0.70 Aa	91.81	1.30 Aa	73.60	0.90 Aa	73.93	1.73 Ba	79.60
Control	8.56 Fb	0	4.96 Da	0	4.13 Da	0	8.50 Db	0

*Means followed by the same letters, upper case in the column and lower case in the row, do not differ significantly from each other using the Scott-Knott test at 5% probability. CV = 25.17%. PGI: Percentage inhibition of mycelial growth. Method 1: circle technique; method 2: paired culture technique; method 3: paired technique with risk in the center of the plate; method 4: fungal culture technique on antagonist culture.

When analyzing the means obtained in the first method tested (circle technique), it is evident that all strains inhibited the mycelial growth of the fungus; however, they showed varying values in terms of the inhibition percentage. Strain UNIFENAS 03-15 exhibited the largest colony diameter, along with a PGI of 35.35%, while the other strains showed inhibition values ranging from 52.77% to 100%. In the second method, which employed the paired culture technique, 19 out of the 21 strains were able to inhibit the fungal mycelial growth. Strains UNIFENAS 02-12 and 03-15 presented colony diameters statistically similar to the control, resulting in the lowest PGI values of 0.65% and 9.91%, respectively. Using the third method, the central streak technique, all strains inhibited the mycelial growth of the pathogen, with strains UNIFENAS 02-11, 03-14, 03-23, 03-33, 03-35, 03-36, and CIAT 899 showing the smallest colony diameter means, indicating higher inhibition. Finally, in the fourth method (fungal culture over antagonist culture), all strains except UNIFENAS 03-15 demonstrated antagonistic potential, with PGI values ranging from 42.74% to 92.16%. Strain UNIFENAS 03-15 showed a colony diameter equivalent to the control and did not inhibit fungal mycelial growth.

Overall, the bacterial strains that presented the smallest colony diameters in at least three of the methods tested were UNIFENAS 03-14, 03-23, 03-27, 03-33, 03-36, and CIAT 899. This finding is of particular interest since a smaller colony diameter is associated with a higher percentage of mycelial growth inhibition and indicates the antagonistic potential of these microorganisms.

Similar results were obtained by Oliveira et al. (2020), who investigated the in vitro antagonistic activity of bacterial strains from the same collection used in the present study against *Pseudocercospora griseola* (Sacc.). That study identified mycelial growth inhibition potential using the circle technique and highlighted that strains UNIFENAS 03-23, 03-27, and 03-36 also demonstrated the ability to inhibit the growth of the causal agent of angular leaf spot, reinforcing the potential of these microorganisms as viable biocontrol agents.

Considering the methods used to evaluate antagonism, it can be inferred that the smallest colony diameters are associated with methods 1 and 4. In a study conducted by Braga Júnior et al. (2017), which investigated the in vitro antagonistic activity of *Bacillus subtilis* strains against three phytopathogenic fungi using the same four methods applied in the present study, the highest inhibition rates of mycelial growth

were observed with the circle and fungal culture over antagonist culture techniques. These findings corroborate the results of this study and highlight the importance of testing different methodologies to evaluate and select potential antagonists.

In the evaluation of the antagonistic activity of the bacterial mixes against the fungus *F. oxysporum* f. sp. *phaseoli*, it was observed that they exhibited an inhibitory effect on the mycelial growth of the pathogen only when subjected to inhibition methods 1, 3, and 4. The results related to the diameter of the fungal colonies and the PGI are presented in (Table 5).

Table 5. Inhibition of the mycelial growth of *Fusarium oxysporum* f. sp. *phaseoli* by a mix of bacterial strains with antagonistic potential.

Treatments	Methods 1		Methods 2		Methods 3		Methods 4	
	Ø (cm)	PGI (%)	Ø (cm)	PGI (%)	Ø (cm)	PGI (%)	Ø (cm)	PGI (%)
Mix 1	0.83 Aa	89.82	2.40 Ab	46.87	2.30 Ab	52.61	2.13 Ab	74.90
Mix 2	0.83 Aa	89.85	2.96 Ab	34.40	3.50 Bb	28.03	1.96 Ab	76.86
Mix 3	0.73 Aa	91.13	2.90 Ab	35.85	2.23 Ab	54.00	2.00 Ab	76.47
Mix 4	1.00 Aa	87.84	2.86 Ab	36.56	3.23 Bb	33.42	1.66 Aa	80.39
Mix 5	0.83 Aa	89.91	2.46 Ab	45.55	3.16 Bb	34.75	3.56 Bb	58.00
Mix 6	0.60 Aa	92.70	2.70 Ab	40.22	2.30 Ab	52.72	2.33 Ab	72.55
Mix 7	0.70 Aa	91.49	3.20 Ab	29.40	3.50 Bb	28.14	2.86 Ab	66.27
Mix 8	1.10 Aa	87.00	2.93 Ab	35.31	3.03 Bb	37.59	2.53 Ab	70.19
Mix 9	1.03 Aa	87.43	3.60 Ab	20.52	3.26 Bb	32.93	3.13 Bb	63.13
Mix 10	0.63 Aa	92.28	3.26 Ab	27.84	3.43 Bb	29.36	2.70 Ab	68.23
Mix 11	0.60 Aa	92.70	3.33 Ab	26.48	3.30 Bb	32.20	1.06 Aa	87.45
Mix 12	0.90 Aa	89.03	2.73 Ab	40.00	1.93 Ab	60.23	2.83 Ab	66.66
Mix 13	1.90 Aa	76.92	4.43 Ab	2.12	3.43 Bb	29.42	4.26 Bb	49.80
Mix 14	0.90 Aa	89.08	3.20 Ab	29.30	2.43 Ab	50.00	2.33 Ab	72.55
Mix 15	0.73 Aa	91.07	3.60 Ab	20.30	3.26 Bb	32.75	1.86 Aa	78.04
Mix 16	1.00 Aa	87.84	3.53 Ab	22.07	3.50 Bb	27.80	3.73 Bb	56.07
Mix 17	1.00 Aa	87.84	3.36 Ab	25.83	3.66 Bb	24.81	2.00 Aa	76.47
Mix 18	0.60 Aa	92.70	3.50 Ab	22.74	3.03 Bb	37.59	2.63 Ab	69.02
Mix 19	0.70 Aa	90.83	3.83 Ab	15.46	3.46 Bb	28.81	3.83 Bb	54.90
Mix 20	0.83 Aa	89.91	3.56 Ab	21.18	3.36 Bb	30.93	2.33 Ab	72.55
Controle	8.23 Bb	0	4.53 Aa	0	4.86 Ba	0	8.50 Cb	0

*Means followed by the same letters, upper case in the column and lower case in the row, do not differ significantly from each other using the Scott-Knott test at 5% probability. CV = 29.73%. PGI: Percentage inhibition of mycelial growth. Method 1: circle technique; method 2: paired culture technique; method 3: paired technique with risk in the center of the plate; method 4: fungal culture technique on antagonist culture.

When subjected to method 1, all bacterial mixes inhibited the mycelial growth of the fungus, with PGI values ranging from 76.92% to 92.70%. On the other hand, none of the bacterial mixes were able to inhibit the pathogen when subjected to the second method, showing colony diameters statistically similar to those of the control treatment. Using the third method, only mixes 1, 3, 6, 12, and 14 inhibited the mycelial growth of the fungus, with PGI values ranging from 50.00% to 60.23%. Finally, in the fourth method, all mixes exhibited an inhibitory effect on the pathogen's growth, with PGI values ranging from 49.80% to 87.45%. Overall, bacterial mixes 1, 3, 6, 12, and 14 showed the smallest colony diameters in at least three of the methods tested.

The strategy of combining bacterial strains with antagonistic potential presents an interesting alternative, as it aims to enhance the action of microorganisms against the phytopathogen through multiple mechanisms. This is particularly relevant due to the inherent complexity of isolating a single strain with such characteristics independently (Santos et al., 2021). In the literature, the approach of using bacterial strains in combination is widely explored. Studies indicate that by uniting specific microorganisms, it is possible to integrate diverse traits that oppose pathogens, which can lead to a significant increase in protection levels (Shen et al., 2020; Santos et al., 2021). Furthermore, this cooperation among microorganisms also shows potential to effectively promote plant growth (Ferreira et al., 2020; Costa et al., 2022).

As observed for the individual strains, when analyzing the methods used to evaluate antagonism by the bacterial mixes, it is also possible to infer that the smallest colony diameters are associated with methods 1 and 4.

The mean PGI values of *F. oxysporum* f. sp. *phaseoli* for the bacterial strains, both individually and in combination, under the four inhibition methods, are presented in (Table 6). The results indicate that the highest levels of inhibition were observed when the strains were subjected to evaluation methods 1 and 4.

Table 6. Average percentage inhibition of the mycelial growth of *Fusarium oxysporum* f. sp. *phaseoli* by bacterial strains, in isolated and combined approaches, submitted to four inhibition methods.

Methods	PGI (%)	
	Isolated	Mix
1	87.65 Aa	81.41 Aa
2	49.37 Ca	27.52 Cb
3	61.50 Ca	35.14 Cb
4	73.59 Ba	66.21 Ba

*Means followed by the same letters, upper case in the column and lower case in the row, do not differ significantly from each other using the Scott-Knott test at 5% probability. CV = 33.47%. PGI: Percentage inhibition of mycelial growth. Method 1: circle technique; method 2: paired culture technique; method 3: paired technique with risk in the center of the plate; method 4: fungal culture technique on antagonist culture.

For clearer visualization, the results are also illustrated in Figure 1.

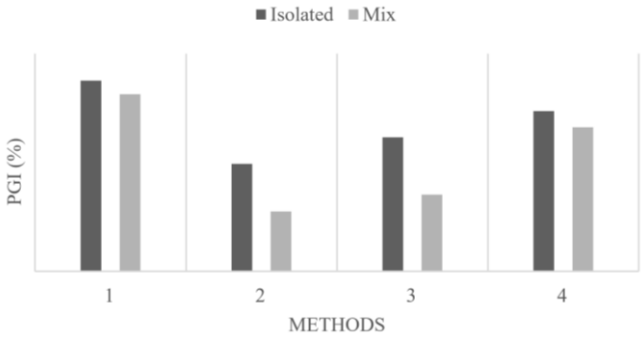

Figure 1. Percentage of mycelial growth inhibition of *Fusarium oxysporum* f. sp. *phaseoli* by bacterial strains, in single and combined approaches, under four inhibition methods. PGI: Percentage inhibition of mycelial growth. Method 1: circle technique; method 2: paired culture technique; method 3: paired technique with risk in the center of the plate; method 4: fungal culture technique on antagonist culture.

Comparing the mean PGI values of the two approaches (isolated and combined strains) shows that the highest inhibition levels were recorded when the strains acted individually. Although studies suggest that combining biocontrol agents results in more effective control than individual applications, there are reports of reduced efficiency due to microorganism incompatibility (Barbosa et al., 2018; Santos et al., 2021). Incompatibility occurs when certain microorganisms cannot coexist or act together because of antagonistic interactions (Izquierdo-García et al., 2020), which can reduce the overall effectiveness of biological control.

In the present study, a preliminary test was conducted to assess the compatibility of the strains, identifying those without visible antagonism. This test only confirms the absence of direct inhibition and does not guarantee cooperative or synergistic action of the strains. When designing biological control strategies, it is essential to consider the characteristics of each microorganism and their potential interactions when applied together (Sruthilaxmi & Babu, 2017; Santos et al., 2021). Therefore, performing compatibility tests is crucial to ensure the effective combined use of antagonists.

Conclusion

The bacterial strains evaluated in this study were effective in inhibiting the mycelial growth of *Fusarium oxysporum* f. sp. *phaseoli* when subjected to the four inhibition methods. However, the bacterial mixes were only effective in three of these methods. The greatest inhibitions were observed when the circle and fungal culture techniques were used on the antagonist culture and when the strains acted in isolation.

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