

Use of Actinobacteria *Streptomyces griseocarneus* for the Control of *Fusarium solani* Fungus in Passion Fruit Crops

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Summary

In passion fruit crops, species of the *Fusarium solani* fungal complex acts as causal agents of collar rot. The symptoms of this disease are characterized by sudden wilting, collapse, and plant death at any stage of development. One of the possible measures to reduce the environmental risk of intensive cultivation is the use of biological controls, which can be found in the form of endophytic microorganisms. In view of this, the present study investigates the use of the endophytic actinobacterium *Streptomyces griseocarneus*, isolate R132, to promote *in vitro* and *in vivo* biological control of *F. solani* isolates in passion fruit seedlings. Two experiments were conducted: 1) The antibiosis assay in paired culture was used to quantify the antagonism between the actinobacterium *S. griseocarneus* R132 and eight isolates of the *Fusarium solani* species complex. 2) The ability of *S. griseocarneus*, isolate R132, to control seven isolates of *F. solani* was tested. In the present study, the *in vitro* antifungal potential of *S. griseocarneus* R132 was observed against all *F. solani* isolates. In the biological control evaluation, the actinobacterium significantly reduced the disease damage compared to untreated plants, suggesting that this bacterium is a potential biological control agent.

Key words

Passiflora edulis Sims, endophytic bacteria, biological control, collar rot, bio-control

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Introduction

In 2021, passion fruit production in Brazil reached 683,993 tons with an average yield of 15.25 tons per hectare (IBGE, 2022), which is considered the world's largest passion fruit production. Furthermore, Brazil is the largest consumer of this fruit (Faleiro and Junqueira, 2016). The species *Passiflora edulis* Sims, commonly referred to as the sour passion fruit, holds the dominant position in national cultivation, accounting for approximately 90% of the total cultivated area (Viana et al., 2016).

Due to its versatile uses, interest in passion fruit has increased in various countries in recent years (Rodrigues et al., 2023). Meeting the increasing global demand for passion fruit hinges on the successful establishment of field plants, a crucial prerequisite that directly correlates with the plants' ability to combat phytopathogenic fungi. These fungi currently stand as the primary contributors to the reduced productivity of passion fruit crops (Fischer and Resende, 2016).

Among the various diseases affecting passion fruit, those induced by fungi belonging to the genus *Fusarium* are noteworthy, particularly species within the *F. solani* complex, which act as causative agents of collar rot disease. The symptoms associated with these diseases manifest as abrupt wilting, collapse, and the eventual demise of plants at any developmental stage (Fischer and Resende, 2016).

One potential strategy for mitigating the harm inflicted by this disease involves the utilization of cultivars that exhibit resistance or tolerance. Nevertheless, it is worth noting that at present, only one registered passion fruit cultivar has demonstrated resistance to collar rot disease, and it goes by the name Maracujá-Tereza (MAPA, 2021). It is a hybrid resulting from the cross between the elite cultivar of *P. edulis* "Sol do Cerrado" and *P. quadrangulares*. Although this hybrid is resistant to collar rot disease, it is sterile, limiting its use to be utilized only as rootstock (Chimello, 2021).

Considering the information provided, an alternative approach to disease management could involve the utilization of biological controls, which are available in the form of endophytic microorganisms (Hamedi and Mohammadipanah, 2014). Endophytic microorganisms are fungi or bacteria that colonize and grow within or between healthy plant tissues, but do not cause harm to the hosts, meaning they are non-pathogenic (Schulz and Boyle, 2006; White et al., 2019).

The use of these microorganisms as biological control agents is possible because they produce enzymes that degrade the fungal cell wall, such as proteases, cellulases, and chitinases, or through the production of substances like siderophores (Melnick et al., 2011; Yu et al., 2011; Zhang et al., 2011). Among the diverse microorganisms utilized as biological control agents, members of the *Streptomyces* genus emerge as notable contenders. They are commonly harnessed for their capacity to combat various phytopathogenic fungi, owing to their ability to produce enzymes that degrade fungal cell walls (Rashad et al., 2017), as well as antibiotics and antifungals (Sabaratnam and Traquair, 2015) or for inducing systemic responses in plants (Salla et al., 2016). Furthermore, these microorganisms find extensive application in promoting plant growth (Toumatia et al., 2016).

Therefore, the present study aimed to investigate the use of the endophytic actinobacterium *Streptomyces griseocarneus* as biological control of *F. solani* isolates in seedlings of the sour passion fruit cultivar "Sol do Cerrado".

Materials and Methods

The experiment was conducted at the Laboratory of Plant Genetic Improvement (LMGV) at the State University of Mato Grosso (UNEMAT), Cáceres campus.

In vitro Assay

The antagonistic relationship between the actinobacterium and isolates from the *Fusarium solani* species complex was quantified using an antibiosis assay in paired culture. For this test, the actinobacterium *Streptomyces griseocarneus*, isolate R132 (ID NCBI: KX618389), previously isolated from the roots of *Paullinia cupana* (Liotti et al., 2017) and selected for higher levels of antagonism in the work of Liotti et al. (2019), was used. The actinobacterium *S. griseocarneus* isolate R132 used in this study was kindly provided by Dr. Marcos Antônio Soares (Federal University of Mato Grosso, Cuiabá, Mato Grosso, Brazil).

First, the inoculum of *F. solani* and the actinobacterium *S. griseocarneus* R132 were prepared. The preserved isolates of *F. solani* and the actinobacterium *S. griseocarneus* R132 on filter paper were cultured on Petri dishes containing Luria-Bertani (LB) culture medium and kept at 25 °C with a 12-hour photoperiod for five days in the biochemical oxygen demand incubator (BOD).

Inoculation

Inoculation was performed with a 5-mm diameter mycelial disk of *Fusarium* spp cultivated in LB culture medium with the isolates placed in the center of a Petri dish containing Luria-Bertani (LB) culture medium. Two disks containing actinobacterium mycelium were deposited diametrically opposed to the *Fusarium* mycelial disk.

The experiment was conducted in a completely randomized design with eight *F. solani* isolates and four replications. A control was added for each isolate (without actinobacterial inoculation).

Following a seven-day incubation period, the mycelial diameter was assessed, and the percentage of fungal growth inhibition was determined using the formula:

$$\% \text{ Inhibition} = [(D - d) \times 100] / D$$

where *D* represents the mycelial diameter (in millimeters) on the control plates, *d* signifies the mycelial diameter that develops in the presence of the actinobacteria.

The obtained data were subjected to analysis of variance, and means were compared using the Tukey test ($P < 0.05$). All analyses were performed using the GENES software (Cruz, 2016).

In vivo Assay

Preparation of Seedlings

Seedlings obtained from cuttings of healthy mature plants of the BRS Sol do Cerrado cultivar were initially placed in 128-cell Styrofoam trays filled with Vivatto substrate and maintained under 50% shade. After a period of three months, these seedlings were transplanted into 500 mL plastic cups containing the same substrate. The selection of this cultivar was based on its attractive attributes, including the production of yellow, large fruits, a pulp yield of approximately 38%, and a high level of productivity, despite its vulnerability to collar rot disease. It also shows tolerance to foliar diseases such as bacterial blight, anthracnose, and virus diseases (EMBRAPA, 2008).

Inoculation

Seven *F. solani* pathogen inocula were used for the inoculation. The preserved isolates on filter paper were situated on Petri dishes filled with PDA (potato-dextrose agar) culture medium, while the R132 actinobacteria were cultured in LB broth (Luria-Bertani). Both cultures were incubated at 25 °C under a 12-hour photoperiod for a duration of seven days within a BOD incubator.

For the inoculation process, 20 µL of the actinobacterial culture was applied to a small 3-mm diameter, made with a sterile blade at the plant's base, approximately two centimeters above the soil. Following this, a 5-mm diameter mycelial disk of the pathogen grown in PDA was placed and secured with PVC plastic over the same wound. The PVC plastic was removed five days after inoculation (DAI) in accordance with the methodology outlined by Fischer et al. (2005). The same procedure was performed for the control, but without the application of the actinobacterial culture.

The experimental design employed in this study was a randomized block design with a factorial arrangement of treatments (2x7). The factors included the presence or absence of co-inoculation with the actinobacterium and seven different *F. solani* isolates. The experiment consisted of three blocks, each containing five plants per plot.

The evaluation of the plants began on the 5th day after inoculation (DAI) and continued every two days until the 33rd DAI or until the point when the plants succumbed to the disease. During these evaluations, the lesions were meticulously measured for both their length and the width of the necrotic area, using a digital caliper. The lesion area (LA, mm²) was calculated using the formula for calculating the area of an ellipse ($\pi CL/4$), where C represented the lesion's length and L represented the lesion's width.

To assess the effectiveness of the actinobacterium as a biological control agent, ten variables pertaining to the lesions were determined, following the description provided by Preisigke et al. (2015).

PS = Survival period in days;

NPM = Number of dead plants at the end of 33 days;

CL = Length of the lesion in mm;

LL = Width of the lesion in mm;

NPL-50% = Number of plants where the lesion reached less than 50% of the circumference at the end of 33 days;

PILA50% = Period in days from inoculation until the lesion reached 50% of the circumference;

PILA100% = Period in days from inoculation until the lesion reached 100% of the circumference;

AACEAL = Area under the curve of lesion area expansion in mm;

AACECL = Area under the curve of lesion length expansion in mm;

AACELL = Area under the curve of lesion width expansion in mm.

The data obtained from the characteristics associated with biological control were subjected to analysis of variance and the means were compared using the Tukey's HSD test at a significance level of $P < 0.05$. All analyses were performed using the GENES software (Cruz, 2016).

Results and Discussion

In vitro Assay

Upon analyzing the data using analysis of variance, a statistically significant effect of the factors was observed ($P < 0.01$), such as the percentage (%) of inhibition and mycelial diameter, of the *in vitro* antibiosis by confrontation between the endophytic actinobacterium *S. griseocarneus* R132 and *Fusarium solani* isolates (Table 1).

Table 1. Antibiosis of endophytic actinobacterium against *F. solani* isolates

Isolates	Growth inhibition (%)	Mycelial diameter (mm)
FSUNEMAT 38	55.96 ± 0.97 ^a	23.94 ± 2.11 ^{bcd}
FSUNEMAT 46	51.30 ± 1.61 ^a	24.67 ± 1.98 ^{bcd}
FSUNEMAT 40	50.91 ± 15.82 ^a	16.26 ± 1.21 ^d
FSUNEMAT 31	49.30 ± 9.80 ^{ab}	21.92 ± 2.21 ^{cd}
FSUNEMAT 25	42.34 ± 1.75 ^{abc}	23.23 ± 1.85 ^{bcd}
FSUNEMAT 12	28.18 ± 9.95 ^{bc}	29.55 ± 5.19 ^{abc}
FSUNEMAT 50	26.52 ± 5.06 ^c	31.48 ± 3.15 ^{ab}
FSUNEMAT 33	22.78 ± 6.25 ^c	37.54 ± 5.71 ^a

Note: The results are expressed as mean ± standard deviation. The means with the same letters in the same column do not differ statistically according to the Tukey's HSD test. ($P < 0.05$)

The actinobacterium *S. griseocarneus* R132 inhibited mycelial growth by at least 20% in all tested isolates of *F. solani* (Table 1 and Fig. 1). This result is in accordance with Rashad et al. (2017) and Liotti et al. (2019), where both authors detected *in vitro* antifungal activity of *S. griseorubens* E44G against *Fusarium oxysporum* f. sp. *lycopersici* and of *S. griseocarneus* R132, *S. prasinopilosus* R199, and *S. acidiscabies* R403 against five species of phytopathogenic fungi, respectively. The highest inhibition rates (> 50%) were observed in isolates FSUNEMAT 38, 46, and 40. Hence, it was determined that there was no correlation between the aggressiveness of the isolates and the percentage of inhibition.

Notably, isolates FSUNEMAT 40 and 46, recognized as the most aggressive *F. solani* isolates in the Plant Genetic Improvement Laboratory (LMGV) collection, as reported by Marostega et al. (2019), exhibited an inhibition rate by *S. griseocarneus* R132 above 50% (Table 1), demonstrating that this bacterium has great inhibition capacity. This observation aligns with findings by El-Mehalawy et al. (2007), who noted that various factors could contribute to the antifungal activity of actinobacteria, including the production of hydrolytic enzymes or hyperparasitism, therefore, even highly aggressive isolates could have activity inhibited by the action of actinobacteria with this capacity. Numerous studies have also reported the antifungal activity of actinobacteria. For instance, *Nocardioides ganghwensis*, *Streptomyces cavourensis*, and *S. parvulus* have been found to inhibit the growth of fungi such as *Aspergillus carbonarius*, *Aspergillus westerdijkiae*, and *Fusarium graminearum* (Salam et al., 2017). *Streptomyces* sp. MM140 has demonstrated inhibitory effects on the growth of *F. oxysporum*, *F. verticilloides*, *Macrophomina phaseolina*, and *Phomopsis* sp., as reported by Solans et al. (2016).

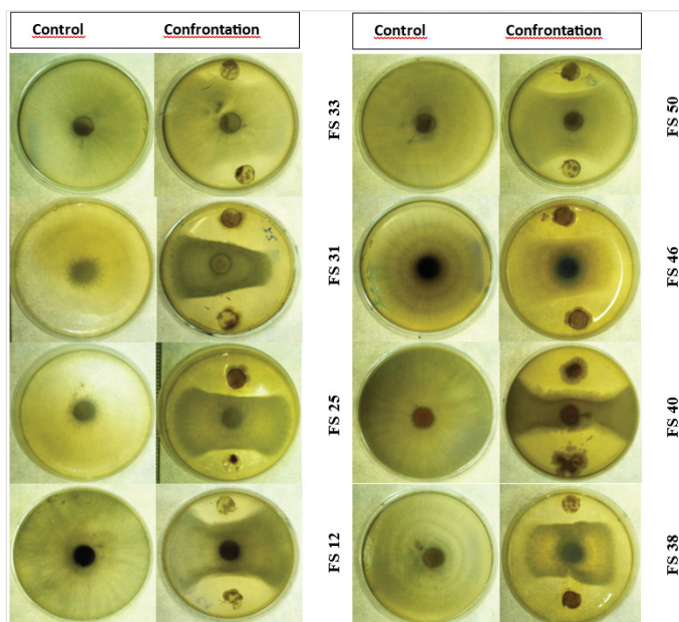


Figure 1. Inhibition of mycelial growth of *F. solani* isolates (FS) by confrontation with endophytic actinobacterium *Streptomyces griseocarneus* isolate R132, compared to untreated control

The results observed in the antibiosis of the actinobacterium to the fungus *F. solani* are promising, as mycelial growth inhibition was independent of the virulence of the isolates towards passion fruit. Thus, the potential of the actinobacterium *S. griseocarneus* R132 as a biological control agent for *F. solani* was identified.

According to Cruz et al. (2000), *S. griseocarneus* is known to produce a range of antimicrobial compounds, including antifungal polyene macrolides and antifungal as well as antibacterial pyrazoloisoquinolines.

In vivo Assay: Biological Control of Collar Rot of Passion Fruit

Upon analyzing the data for lesion characteristics used as variables in the analysis of variance, a significant effect was observed, with a probability of 1%, for the factor "Bacterium", that is, the comparison of seedlings treated and not treated with Bacterium, in the variables PS, CL, AACEAL, and AACELL. The variables NPM and LL showed a significant effect at a probability of 5%. The factor "Isolates", that is, the comparison of different isolates of *F. solani*, to the effect of treatment with the bacterium, demonstrated a statistically significant effect at a 1% level of significance in nine out of the ten analyzed variables, with the only exception being the variable AACECL, which exhibited significance at a 5% probability level. This concerned the assessment of the biological control capacity of *S. griseocarneus* R132 against *F. solani*.

Moreover, the interaction between "Bacterium × Isolates" exhibited a significant effect for the variables PS (Survival period in days), CL (Length of the lesion in mm), and AACEAL (Area under the curve of lesion area expansion in mm) at a 1% probability level. However, there was no observed significant effect for the other variables based on the F-test.

Out of the ten evaluated variables, six showed a significant difference for the factor "Bacterium," indicating that the results were influenced by the co-inoculation of the endophytic actinobacterium *S. griseocarneus* R132. In plants with co-inoculation, a reduction in the damages caused by the action of the fungus *F. solani* was observed (Table 2).

Six days after the inoculation of *F. solani* isolates in sour passion fruit seedlings (*P. edulis*), the first deaths occurred due to disease damage. Overall, the co-inoculation of the actinobacterium *S. griseocarneus* R132 with the phytopathogenic fungus significantly reduced the damage caused by the disease compared to untreated plants infected only with *F. solani* isolates.

The most virulent isolates on *P. edulis* seedlings were FSUNEMAT 40 and 46, confirming the results found by Marostega et al. (2019). Although isolate 40 showed a lower PS with the co-inoculation of the actinobacterium from 8 to 6 days, the other variables showed a significant reduction in damage caused by the *F. solani* fungus, such as a decrease in NPM from 2 to 1.66. This reduction in damage caused by *F. solani* was also observed in isolate 46, which showed an increase in PS from 5.5 to 20.33 and a reduction in NPM from 1.66 to 1 with the actinobacterium inoculation. Additionally, increases in PS were observed in isolates FSUNEMAT 50, 38, and 31 (Table 3). One potential explanation for the biological control of *F. solani* is that endophytic microorganisms, under both biotic and abiotic stress conditions, can trigger the activation of resistance genes in plants, as proposed by Majid et al. (2016).

No significant difference was observed with the Tukey's test ($P < 0.05$) between plants treated and non-treated with the actinobacterium within each isolate, only between the isolates (Table 3).

In general, despite the introduction of the actinobacterium, the aggressiveness of the isolates remained consistent with the findings reported by Marostega et al. (2019).

Table 2. Means values for the factor "Bacterium" in the *in vivo* confrontation antibiosis between endophytic actinobacterium and isolates of *F. solani*

Treatments	PS	NPM	CL	LL	AACEAL	AACELL
with the bacterium	27.00 ^a	0.47 ^b	18.03 ^b	4.12 ^b	41.62 ^b	2.51 ^b
without the bacterium	23.64 ^b	0.71 ^a	30.86 ^a	6.77 ^a	81.06 ^a	3.61 ^a

Note: Means in a row with the same letters in the same column do not differ statistically by Tukey's HSD test ($P < 0.05$); PS = Survival period in days; NPM = Number of dead plants at the end of 33 days; CL = Length of the lesion in mm; LL = Width of the lesion in mm; AACEAL = Area under the curve of lesion area expansion in mm and AACELL = Area under the curve of lesion width expansion in mm

This pattern indicated that isolates 40 and 46 remained the most aggressive, isolate 31 exhibited intermediate aggressiveness, while the remaining isolates displayed lower levels of aggressiveness. Notably, plants that were not subjected to fungal inoculation (control) remained free from disease symptoms, underscoring that the *F. solani* isolates were indeed the primary causative agents of the observed lesions. At the end of the experiment, in 100% of the seedlings inoculated with *F. solani*, it was possible to re-isolate and identify the fungus, hence Koch's postulates were satisfied.

The interaction of the factors "Bacteria" × "Isolates" was observed for the variables PS, CL, and AACEAL. The greatest differences on PS were observed in isolates FSUNEMAT 46, 50, and 31, with an increase of means from non-inoculated controls to treatments treated with actinobacteria as follows: from 5.50 to 20.33, from 28.66 to 33 and from 23.66 to 33, respectively (Table 4).

The variable CL showed the highest significant difference, where among the seven analyzed isolates, only the FSUNEMAT 33 isolate did not show a significant difference between plants inoculated and not inoculated with the actinobacterium. The results observed for this variable indicate that in all isolates with statistically significant differences, the bacterium reduced the damage caused by the *F. solani* fungus.

Regarding the variable AACEAL, a significant difference was observed between plants inoculated and not inoculated with the actinobacterium for the isolates FSUNEMAT 40, 46, 50 and 31 (Table 4). For the variable PS, significant differences were only observed for isolates 40 and 46 compared to the other isolates when co-inoculated with the actinobacterium (Table 4).

The variable AACEAL, similarly to CL, showed greater differences with plants not inoculated with actinobacterium. Isolate 40 obtained the highest value with 222.78 for AACEAL, followed by isolate 31 with 78.61. For plants without the actinobacterium, a significant difference was observed only between isolate 31 and the other isolates.

Collar rot remains a significant issue resulting in substantial losses within passion fruit crops, as outlined by Fischer and Resende (2016). The current study has shown that *S. griseocarneus* R132 effectively contributes to the biological control of *F. solani*, corroborating the findings of Liotti et al. (2019). Liotti and colleagues demonstrated the potential of the actinobacterium *S. griseocarneus* R132 in inhibiting the mycelial growth of various phytopathogens, including *Fusarium oxysporum*, which is responsible for wilt disease in *Capsicum* spp.

Table 3. Means of values of characteristics of lesions developing on plants inoculated and non-inoculated with the actinobacterium for the *in vivo* confrontation antibiosis test between endophytic actinobacterium *Streptomyces griseocarneus* isolate R132 and *F. solani* isolates

Treatments ¹	PS	NPM	CL	LL	NPL-50%	PILA-50%	PILA-100%	AACELL	AACECL	AACEAL
FSUNEMAT40	7.33 ^c	1.83 ^a	41.70 ^a	10.65 ^a	0.16 ^c	6.25 ^c	6.66 ^c	5.02 ^a	17.15 ^{ab}	129.64 ^a
FSUNEMAT33	33.00 ^a	0.00 ^c	22.71 ^{ab}	6.72 ^{ab}	2.00 ^a	33.00 ^a	33.00 ^a	2.81 ^{abc}	17.65 ^{ab}	45.85 ^b
FSUNEMAT46	12.91 ^b	1.33 ^a	21.63 ^c	3.78 ^b	0.66 ^{bc}	15.00 ^b	15.83 ^b	2.39 ^c	12.57 ^b	45.91 ^b
FSUNEMAT50	30.83 ^a	0.16 ^{ab}	20.43 ^c	4.10 ^b	1.83 ^a	30.83 ^a	30.83 ^a	2.40 ^c	15.45 ^{ab}	40.66 ^b
FSUNEMAT38	31.83 ^a	0.16 ^{ab}	17.89 ^c	3.23 ^b	1.83 ^a	31.83 ^a	31.83 ^a	2.45 ^{ab}	16.58 ^{ab}	31.43 ^b
FSUNEMAT25	33.00 ^a	0.00 ^c	16.62 ^c	2.20 ^b	2.00 ^a	33.00 ^a	33.00 ^a	1.61 ^c	13.07 ^b	20.93 ^b
FSUNEMAT31	28.33 ^a	0.66 ^b	30.14 ^b	7.45 ^{ab}	1.00 ^b	18.33 ^b	26.50 ^a	4.73 ^{ab}	22.72 ^a	114.99 ^a
Control	33.00 ^a	0.00 ^c	13.75 ^c	2.63 ^b	2.00 ^a	33.0 ^a	33.0 ^a	1.14 ^c	12.00 ^b	10.74 ^b

Note: Means followed by the same letter in the same column do not differ statistically by Tukey's HSD test ($P < 0.05$); ¹ Treatments: *F. solani* isolates in the column. PS = Survival period in days; NPM = Number of dead plants at the end of 33 days; CL = Length of the lesion in mm; LL = Width of the lesion in mm; NPL-50% = Number of plants where the lesion reached less than 50% of the circumference at the end of 33 days; PILA50% = Period in days from inoculation until the lesion reached 50% of the circumference; PILA100% = Period in days from inoculation until the lesion reached 100% of the circumference; AACECL = Area under the curve of lesion length expansion in mm; AACELL = Area under the curve of lesion width expansion in mm and AACEAL = Area under the curve of lesion area expansion in mm

Table 4. Interaction of the factors “Bacterium” × “Isolates” for the *in vivo* confrontation antibiosis test between endophytic actinobacterium *S. griseocarneus* isolate R132 and *F. solani* isolates. Plants inoculated with the actinobacterium (C) and without the actinobacterium (S).

Treatments	PS		CL		AACEAL	
	C	S	C	S	C	S
FSUNEMAT40	6.00 ^{aC}	8.66 ^{aC}	22.92 ^{bA}	60.49 ^{aA}	36.50 ^{bB}	222.78 ^{aA}
FSUNEMAT33	33.00 ^{aA}	33.00 ^{aA}	22.59 ^{aA}	22.84 ^{aC}	38.85 ^{aB}	52.85 ^{aBC}
FSUNEMAT46	20.33 ^{aB}	5.50 ^{bC}	16.71 ^{bA}	26.56 ^{aBC}	18.23 ^{bB}	73.55 ^{aBC}
FSUNEMAT50	33.00 ^{aA}	28.66 ^{aAB}	14.12 ^{bA}	26.71 ^{aBC}	13.09 ^{bB}	68.23 ^{aBC}
FSUNEMAT38	30.66 ^{aA}	33.00 ^{aA}	14.13 ^{bA}	21.65 ^{aC}	22.57 ^{aB}	40.27 ^{aBC}
FSUNEMAT25	33.00 ^{aA}	33.00 ^{aA}	12.38 ^{bA}	20.86 ^{aC}	10.73 ^{aB}	31.12 ^{aC}
FSUNEMAT31	33.00 ^{aA}	23.66 ^{bB}	23.37 ^{bA}	36.91 ^{aB}	151.37 ^{aA}	78.61 ^{bB}

Note: Means followed by the same letter do not differ statistically from each other. lowercase in the column and uppercase in the row. by Tukey's HSD test ($P < 0.05$); PS = Survival period in days; CL = Length of the lesion in mm; AACEAL = Area under the curve of lesion area expansion in mm

However, as suggested by Liotti et al. (2019), one hypothesis is that *S. griseocarneus* R132 exerts its action through plant defense mechanisms. These mechanisms may involve the induction of synthesis and secretion of enzymes by the plant inoculated with actinobacteria, capable of breaking down the cell walls of fungi, together with the production of antifungal compounds that hinder the mycelial growth of phytopathogens.

There are several ways in which endophytic microorganisms can inhibit the growth and fitness of pathosystems (White et al., 2018). The main means is through mechanisms like direct antagonism by competing with pathogens for space and nutrients, as well as through the production of antimicrobial metabolites (Hardoim et al., 2015; Irizarry and White, 2017).

Several studies suggest that endophytic microorganisms provide defense to host plants against pathogens and pests (Ongena and Jacques, 2008; Hardoim et al., 2015; Gond et al., 2015). In addition to antibiosis and competition, according to Hoster et al. (2005), the action of hydrolytic enzymes may have contributed to the biological control of *S. griseocarneus* R132 against *F. solani* isolates.

The outcomes obtained in this study are intriguing, as they confirm the antagonistic efficacy of the actinobacterium through assessments conducted both *in vitro* and *in vivo*. Therefore, these results demonstrate that the actinobacterium used is a promising agent for biocontrol of *F. solani* in passion fruit.

Conclusion

In this study, the *in vitro* antifungal potential of actinobacteria *S. griseocarneus* R132 was observed against all tested isolates of *F. solani*. In the biological control evaluation *in vitro*, the actinobacteria significantly reduced the damage caused by the fungus compared to untreated passion fruit seedlings, suggesting that this bacterium is a potential biological control agent. Therefore, the use of this bacterium may represent a promising strategy for managing of Collar rot disease caused by the fungus

F. solani in passion fruit plantations. Further research and field trials are warranted to fully validate its effectiveness and safety as a biological control agent.

CRedit Authorship Contribution Statement

Antonio Marcos Chimello: performed most of the experiments, analyzed the data and wrote the manuscript. **Marcos Antônio Soares:** donated actinobacteria. **Sabrina Cassaro:** helped with the conceptualization. **Maria do Socorro Bezerra de Araújo:** contributed to editing the manuscript. **Thiago Alexandre Santana Gilio:** contributed to editing the manuscript. **Kelly Lana Araújo:** contributed to editing the manuscript. **Leonarda Grillo Neves:** conceived the project and supervised the work.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this article.

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acs89_15