

# Biocontrol of rice sheath blight with microorganisms obtained in rice cultivated soils

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**ABSTRACT:** Actinobacteria of the genus *Streptomyces* are part of the soil microbiota from rice-growing areas and along with other microorganisms, such as *Trichoderma* spp., combine to act as natural enemies against the destructive soil-borne pathogen *Rhizoctonia solani*, causal agent of rice sheath blight disease. In this study, seven actinobacteria of the genus *Streptomyces* and three fungi of the genera *Trichoderma* and *Purpureocillium* were isolated from soils cultivated with rice using the serial dilution method. *Streptomyces* spp. M2A2 was selected for its ability to significantly reduce the *in vitro* growth of *R. solani* by 52% after 96 h by antibiosis in dual culture, while in the control treatment the mycelial growth was 100%. Furthermore, biocontrol efficacy of treated plants of the susceptible cultivar Fedearroz 68 with actinobacteria was confirmed and the onset of symptoms were delayed up to 14 days, compared to the control treatment. Rice plants treated with *Streptomyces* spp. M2A2 showed lesions of *R. solani* reaching 0.7% of the plant height, the effectiveness of this treatment was similar to the difenoconazole treatment, whereas in the control treatment, the lesions covered 34% of the plant height. When compared to the antagonist fungus *Trichoderma* spp. M2H1, *Streptomyces* spp. M2A2 presented a better performance of biological control. The results clearly demonstrated that *Streptomyces* spp. M2A2 isolate from soils of rice growing areas has biocontrol efficiency against *R. solani* and therefore can be a promising biocontrol agent.

**Key words:** actinobacteria, *Streptomyces* spp., fungal phytopathogen, *Oryza sativa*.

## INTRODUCTION

Different fungi affect the quality and quantity of the production of the rice crop *Oryza sativa* (Cuevas and Higuera 2018). One of which is *Rhizoctonia solani*, a causal agent of rice sheath blight, one of the most destructive soil-borne pathogens in rice-growing regions (El-Shafey et al. 2019; Jia et al. 2013).

Out of the disease management strategies, pest management is considered the most common and efficient component (Mahmood et al. 2017), however, modern agricultural systems point to the search for alternatives that guarantee crop protection and sustainable production without negative effects (Kunova et al. 2016). Biocontrol agents constitute a sustainable control method of plant pathogens (Massart et al. 2015).

In nature, plants are associated with microbial communities (Berg et al. 2017; Massart et al. 2015) that are recruited by the plant from microbiota present around the roots (Vandenkoornhuysse et al. 2015), that guarantees soil health and plant protection product plant-microorganism interaction (Vandenkoornhuysse et al. 2015). In search of agriculturally important microorganisms, the soil is an important source of natural enemies. An example is the abundance and prevalence of *Streptomyces* species in soil suppressive to *R. solani* (Cordovez et al. 2015).

Previous studies showed that *Streptomyces* can be a relevant biocontrol agent of different soil borne pathogens, including *R. solani* (Singh et al. 2016; Tamreihao et al. 2016; Wu et al. 2019), *Fusarium oxysporum f. sp. lycopersici* (Abbasi et al. 2019)



and *Phytophthora capsici* (Park et al. 2012). Additionally, *Streptomyces* species demonstrate biocontrol efficacy in different crops, including rice (Boukaew et al. 2013; Prabavathy et al. 2006; Wu et al. 2019; Yang et al. 2017), tomato (Goudjal et al. 2014) and cucumber (Ohike et al. 2018).

Several mechanisms can be involved in the biological control provided by *Streptomyces*, such as antibiosis. Volatile organic compounds can inhibit the mycelial growth of soil-borne pathogens (Boukaew et al. 2013; Cordovez et al. 2015), likewise, the production of antibiotics by *Streptomyces* spp. affects the development of pathogens like *R. solani* (Wu et al. 2019).

Antagonistic activity of *Streptomyces* species has also been associated with the release of the cell wall degrading enzymes 1,3- $\beta$ -D-glucanase (Park et al. 2012) and chitinase and  $\beta$ -1,3 glucanase against *R. solani*. In addition, *Streptomyces* species can provide control via induced systemic resistance (ISR) against *R. solani* (Patel et al. 2018; Singh et al. 2016) and *Fusarium oxysporum* f. sp. *lycopersici* (Abbasi et al. 2019).

Considering the importance of soil microorganisms in plant fitness and plant protection, the main goal of the present study was to select microorganisms isolated from rice-growing areas with *in vitro* and *in vivo* biocontrol potential against *R. solani*.

## METHODOLOGY

The experiments were carried out in two phases, firstly *in vitro* antagonism assay of microorganisms against *R. solani* and secondly *in vivo* biocontrol trials in susceptible rice plants.

As part of the *in vitro* research, seven actinobacteria of the genus *Streptomyces*, according to morphological characteristics (M1A1, M2A1, M2A2, M2A3, M3A1, M3A2, M3A3), and three fungi of the genera *Trichoderma* (M2H1) and *Purpureocillium* (M3H1 and M3H2) from soil rice-growing areas in Villavicencio-Meta, Colombia were isolated using the serial dilution method (Faheem et al. 2015). The pathogen *R. solani* was isolated from rice plants with symptoms of sheath blight disease, following the tissue disinfection methodology by Ramos-Molina et al. (2016).

The *in vitro* antifungal activity of strains against *R. solani* was performed using the dual culture technique in potato dextrose agar (PDA) (Tagele et al. 2018). Mycelium discs (5 mm in diameter) of each fungal isolation and *R. solani* were placed on the opposite side of each Petri dish 1 cm away from the edge. With the actinobacteria, a stretch mark was made on the opposite side of the pathogen 72 h earlier (Zhang et al. 2016). The absolute control consisted of *R. solani* and the chemical control comprised PDA supplemented with the fungicide difenoconazole 3 mL/L.

The calculation of *in vitro* biocontrol efficacy was carried out using the formula by Ezziyyani et al. (2004) (Eq. 1), which determines the percentage of pathogen growth inhibition (PGI):

$$\text{PGI} = (R1 - R2)/R1 \times 100 \quad (1)$$

where R1 is the radius of the control pathogen and R2 is the radius of the pathogen in confrontation with each isolate.

The microorganism (M2A2) that provided the biocontrol of *R. solani* was identified at the genera level by molecular analysis. The ribosomal gene 16S region was used for the actinobacteria. The Ribosomal Database Project (RDP) allowed for the determination of the genus.

*In vivo* biocontrol trials with the cultivar Fedearroz 68 susceptible to *R. solani* were carried out with microorganisms *Streptomyces* spp. M2A2 and *Trichoderma* spp. M2H1, which showed *in vitro* antagonistic potential. In addition, fungi *Purpureocillium* spp. M3H1 and *Purpureocillium* spp. M3H2 that presented PGI values greater than 49% were also evaluated.

The inoculum of antagonistic microorganisms and pathogen *R. solani* were multiplied in sterile rice grains (Uppala and Zhou 2018). The substrate used in the experiment was collected in a nonoperated area and treated with 10% formalin (Sharma et al. 1990; Irigoyen and Vela 2005). Plastic bags of polyethylene with a capacity of 5 kg were used.

Each microorganism, fungi and actinobacteria was adjusted to a concentration of  $1 \times 10^7$  spores\*mL<sup>-1</sup>. A 100 mL volume of the suspension was applied to the substrate three days before, at the time 'Fedearroz 68' was planted and later at 33 and

62 days after sowing. The inoculum of *R. solani* was applied to the substrate as an 80 mL mycelium suspension per plant at the time of planting.

At the onset of the symptoms, the incidence of the disease at 95, 102 and 109 days and the rate of the disease at 109 days was determined following the formula presented in Eq. 2 (Sharma et al. 1990).

$$\text{Disease index (\%)} = [\text{Highest point where the lesion was observed (cm)} / \text{plant height (cm)}] \times 100 \quad (2)$$

The efficacy of the biocontrol was determined by applying the formula presented in Eq. 3 (Yu et al. 2017):

$$\text{Efficacy of biocontrol} = [(\text{Control disease index} - \text{disease index in treated plants}) / \text{control disease index}] \times 100\% \quad (3)$$

*In vitro* tests were performed in a completely randomized design and independently for fungal isolates and actinobacteria, with seven repetitions (Petri dishes) per treatment. At the *in vivo* tests, the same design was used with seven repetitions. Variance analysis (ANOVA) and the least significant difference (LSD) test  $p \leq 0.05$  were performed for comparison of means using the Sisvar software version 5.6. Biocontrol efficiency percentage values were transformed with Aseno.

## RESULTS AND DISCUSSION

The results showed *in vitro* inhibiting the mycelium growth of *R. solani* with *Streptomyces* spp. M2A2 and *Trichoderma* spp. M2H1 demonstrating antifungal activity (Table 1 and Fig. 1).

**Table 1.** Percentage of growth inhibition (PGI) *in vitro* of *R. solani* by fungal and actinobacterial isolates 96 h after the start of the confrontation.

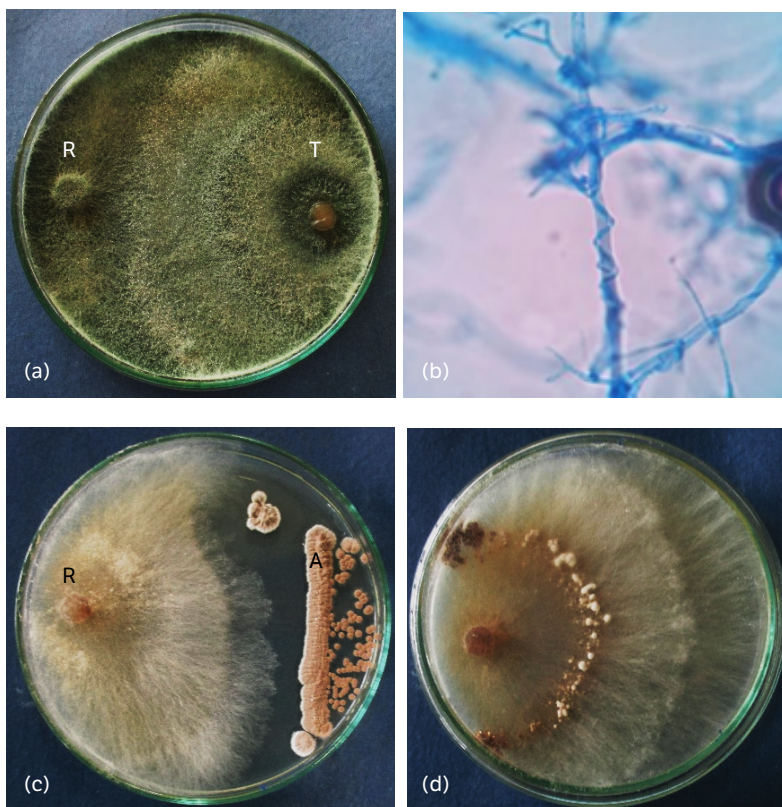
Fungi	
Treatment	PGI 96 h
<i>Purpureocillium</i> spp. M3H1	49 <sup>a</sup> c
<i>Purpureocillium</i> spp. M3H2	52 <sup>c</sup> b
<i>Trichoderma</i> spp. M2H1	78b
Difenoconazole	100a
Actinobacteria	
Treatment	PGI 96 h
<i>Streptomyces</i> spp. M2A1	21e
<i>Streptomyces</i> spp. M2A3	22e
<i>Streptomyces</i> spp. M3A2	26de
<i>Streptomyces</i> spp. M3A1	33cd
<i>Streptomyces</i> spp. M3A3	32cd
<i>Streptomyces</i> spp. M1A1	39c
<i>Streptomyces</i> spp. M2A2	52b
Difenoconazole	100a

<sup>a</sup> PGI: Percentage of growth inhibition. PGI = [(radius of the control pathogen - radius of the pathogen in confrontation)/radius of the control pathogen] × 100.

<sup>b</sup> Different letters within the same column indicate significant differences according to the LSD test ( $p \leq 0.05$ ).

The actinobacteria *Streptomyces* spp. M2A2 significantly inhibited the mycelium growth of *R. solani* (data not shown) 24 h after the dual culture started and managed to maintain its antagonistic efficacy for up to 96 h with 52% inhibition of the growth of *R. solani*, which was significantly higher than the other actinobacteria isolates that ranged between 21 and 39% (Table 1).

On the other hand, after just 72 h a significant antagonistic effect *in vitro* against *R. solani* of fungus *Trichoderma* spp. M2H1 was observed (data not shown). At 96 h, the percentage of *R. solani* inhibition was 78% significantly higher than *Purpureocillium* spp. M3H1 and *Purpureocillium* spp. M3H2 with values of 49 and 52%, respectively (Table 1). The mechanisms of action of antagonistic microorganisms varied: antibiosis activity by *Streptomyces* spp. M2A2 against *R. solani* was confirmed through mycelium growth inhibition (Fig. 1c) and *Trichoderma* spp. M2H1 competed for space and subsequently mycoparasites the *R. solani* hyphae (Fig. 1a, b).



**Figure 1.** *In vitro* growth inhibition of *R. solani* by antagonistic microorganisms 96 h after the start of the confrontation. (a) *Trichoderma* spp. M2H1 vs. *R. solani*. (b) *Trichoderma* spp. M2H1 growing around the hyphae of *R. solani*. (c) *Streptomyces* spp. M2A2 creating a zone of inhibition against *R. solani*. (d) *R. solani* at 96 h. A: Actinobacteria. R: *R. solani*. T: *Trichoderma* spp.

In this work, the production of antifungal compounds by *Streptomyces* spp. M2A2 is likely to have caused antibiosis activity, which significantly inhibited growth *in vitro* of *R. solani* in dual culture (Fig. 1c). Previous reports confirm that members of the *Streptomyces* genus are recognized in producing different antifungal compounds. For instance, Cordovez et al. (2015) confirmed the inhibition of hyphal growth of *R. solani* by the volatile organic compounds (VOCs) methyl 2-methylpentanoate and 1,3,5-trichloro-2-methoxybenzene, produced by *Streptomyces* isolates obtained from a *Rhizoctonia*-suppressive soil. In another study, Boukaew et al. (2013) showed that the VOCs produced by *Streptomyces philanthis* RM-1-138 caused damage at cellular level by disrupting the cell walls, the formation of pores and distortions of the fungal hyphae, which drastically influenced the development of *R. solani*. In addition to VOCs, the antibiotic antifungalmycin (3-methyl-3,5-amino-4-vinyl-2-pyrone, C<sub>6</sub>H<sub>7</sub>O<sub>2</sub>N) produced by *Streptomyces* spp. N2 affects the mycelial growth and sclerotia germination of the causal agent of rice sheath blight (Wu et al. 2019).

Furthermore, to the production of secondary metabolites, other *Streptomyces* species are known for the production of cell wall degrading enzymes against soil borne pathogens. Park et al. (2012) confirmed that antagonistic specie *Streptomyces torulosus* produced hydrolytic enzyme like 1,3-β-d-glucanases that degrade the cell wall of *Phytophthora capsici* and *R. solani* and cause the inhibition of hyphal growth. Similarly, Tamreihao et al. (2016) reported antagonistic activity against *R. solani* by

chitinase,  $\beta$ -1,3-glucanase and  $\beta$ -1,4-glucanase from *Streptomyces corchorusii*. In the same way, Yandigeri et al. (2015) confirmed the biocontrol efficacy against *R. solani* by chitinase enzyme production ability of *Streptomyces vinaceusdrappus* S5MW2.

In the *in vitro* results, the *Trichoderma* isolate M2H1 showed typical mycoparasitic characteristic, growing over the *R. solani* hyphae (Fig. 1b), which is common for this biocontrol agent (Chen et al. 2016). In the plant trials, however, the stronger suppression was obtained with the *Streptomyces* isolate M2A2 (Table 2), showing that this microorganism may have a greater potential as to provide the biocontrol of rice sheath blight.

**Table 2.** Sheath blight index and efficacy of control of fungal and actinobacterial isolates in 'Fedearroz 68' plants at 109 days after sowing (das).

Treatment	Disease index (%)	Biocontrol efficacy (%)
<i>Streptomyces</i> spp. M2A2	0.7 <sup>b</sup> c <sup>a</sup>	98 <sup>c</sup> a
Difenoconazole	3c	90ab
<i>Trichoderma</i> spp. M2H1	14b	63bc
<i>Purpureocillium</i> spp. M3H2	22ab	41c
<i>Purpureocillium</i> spp. M3H1	22ab	43c
Control	34a	-----

<sup>a</sup> Values with different letters in the same column indicate significant difference according to the LSD test ( $p \leq 0.05$ ). <sup>b</sup> Disease index (%): [highest point where the lesion was observed (cm)/plant height (cm)]  $\times$  100. <sup>c</sup> Biocontrol efficacy = [(control disease index - disease index in treated plants)/control disease index]  $\times$  100%. <sup>b-c</sup> Values transformed with Aseno.

The preventive treatment of the soil with *Streptomyces* spp. M2A2 and, during the development of the rice plants, increased the incubation period of *R. solani* and reduced the progress of rice sheath blight disease. This effectiveness of biocontrol using actinobacteria was similar to the chemical treatment Difenoconazole. *Streptomyces* spp. M2A2 delayed the onset of symptoms until 109 days after sowing (DAS) in 'Fedearroz 68' plants, 14 days after the control treatment and isolates *Purpureocillium* spp. M3H2, *Purpureocillium* spp. M3H1 and, finally, the chemical difenoconazole where symptoms appeared at 95 DAS. Similar results were obtained showing an increase in the incubation period of the disease with *Trichoderma* spp. M2H1, with symptoms appearing at 102 DAS and 7 days after control treatment (data not shown).

These results are in concurrence with Ohike et al. (2018), whom selected *Streptomyces lavenduligriseus* KT from 53 actinobacteria isolated from soil samples, which demonstrated a growth inhibition effect against *R. solani in vitro*, subsequently in the treated plants (*Cucumis sativus* L), the appearance of the symptoms of damping off was three days delayed.

The progression of *R. solani* lesions in the plants treated with *Streptomyces* spp. M2A2 reached 0.7% of the plant height, a significantly low percentage and similar to the treatment with difenoconazole. The efficacy percentages for these two treatments were 98 and 90%, respectively. Furthermore, *Trichoderma* spp. M2H1 showed lesions that reached 14% plant height and 63% efficacy. In contrast, the control plants showed sheath blight lesions that reached 34% plant height (Table 2).

The results showed that the treatment of susceptible rice plants with *Streptomyces* spp. M2A2 increases the incubation period of the pathogen and reduces disease progression in treated plants. These results are in agreement with various reports which show that disease control activity can be influenced by different mechanisms that actinobacteria employs. For example, Wu et al. (2019) confirmed that the antifungal mycin N2 (3-methyl-3,5-amino-4-vinyl-2-pyrone, C<sub>6</sub>H<sub>7</sub>O<sub>2</sub>N) produced by *Streptomyces* spp. guarantees healthy plant tissues, which is reflected by a reduced sheath blight severity as a consequence that inhibits sclerotia germination of *R. solani*. On the other hand, Boukaew et al. (2013) showed that volatile compounds of *S. philantheri* cause toxic effects against *R. solani*, which reduced the incidence and severity of rice leaf blight without having direct contact with rice plant tissues.

In addition, there are some reports available on *Streptomyces* spp. as a biocontrol agent via induced systemic resistance. *Streptomyces*-treated plants provide disease protection against *R. solani* associated with enhancing plant defense responses more efficiently, product the priming of plant defenses in response to treatment with *Streptomyces* spp. in the plant (Singh et al. 2016). Furthermore, the activation of phenylalanine ammonia lyase via and induction of ERF1 expression transcription factor involved in ET/JA in tomato by antagonistic *Streptomyces* isolates confirmed induced resistance against *F. oxysporum* f. sp. *lycopersici* (Abbasi et al. 2019).

The confirmation of the genus of isolate M2A2 with the RDP classifier was able to determine that the sequence corresponded to the genus *Streptomyces* with 100% confidence (GenBank accession number MN428020).

## CONCLUSION

In conclusion, the results showed that the soil is an important source that is beneficial to the microorganism. *Streptomyces* M2A2 disease was selected and demonstrated *in vitro* and *in vivo* biocontrol efficacy against *R. solani* causal agent of rice sheath blight. In addition to inhibiting the growth of *R. solani*, *Streptomyces* spp. M2A2 delayed the onset of symptoms and affected the progress of the pathogen in susceptible plants of the cultivar Fedearroz 68 without differences with the difenoconazole treatment and producing better results than *Trichoderma* spp. M2H1 isolate. The results highlight the possibilities for using *Streptomyces* spp. in *R. solani* management.

## AUTHORS' CONTRIBUTION

Conceptualization: Zambrano E. C., Parra A. S. and Ortiz A. M. M.; Methodology: Zambrano E. C., Parra A. S. and Ortiz A. M. M.; Investigation: Zambrano E. C. and Ortiz A. M. M.; Writing – Original Draft: Zambrano E. C. and Ortiz A. M. M.; Writing – Review and Editing: Ortiz A. M. M.; Funding Acquisition: Zambrano E. C., Parra A. S. and Ortiz A. M. M.; Resources: Parra A. S. and Ortiz A. M. M.; Supervision: Ortiz A. M. M.

## DATA AVAILABILITY STATEMENT

All dataset were generated or analyzed in the current study.

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