

## Biological control of *Corynespora cassicola* and *Drechslera tritici-repentis*

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### ABSTRACT

Chemical control is the most widely used method for disease management in significant crops such as soybeans and wheat. However, for a few years biological control has gained prominence. Thus, we evaluated the antagonism of bacteria *Pseudomonas fluorescens*, *Pantoea agglomerans*, and *Bacillus* sp. on the phytopathogens *Corynespora cassicola* and *Drechslera tritici-repentis*, previously isolated from soybean and wheat leaves, respectively. The experiments were carried out under controlled conditions at the Phytobacteriology Laboratory of the Faculty of Agronomy and Veterinary Medicine (FAVM), University of Passo Fundo (UPF), Rio Grande do Sul, Brazil. The treatments were: T1: *P. fluorescens* + pathogen; T2: *P. agglomerans* + pathogen; T3: *Bacillus* spp. + pathogen, and T4: pathogen (control). In each experiment (*C. cassicola* and *D. tritici-repentis*), a completely randomized design with six replications was used. The data were submitted to linear regression analysis, obtaining the daily increase rate (slope). The final time data was submitted to the ANOVA, and the means were compared by the Tukey test ( $P < 0.05$ ). *P. fluorescens*, *P. agglomerans*, and *Bacillus* sp. reduced mycelial growth by 74 and 87% of *C. cassicola* and *D. tritici-repentis*, respectively. Although this study was carried out under *in vitro* conditions, it can serve as a basis for other biological control studies, especially about the management of leaf spots caused by *C. cassicola* and *D. tritici-repentis*, under field conditions.

**Keywords:** *Pseudomonas fluorescens*, *Pantoea agglomerans*, *Bacillus* sp., Mycelial growth inhibition.

### Controle biológico de *Corynespora cassicola* e *Drechslera tritici-repentis*

#### RESUMO

O controle químico é o método mais usado no manejo de doenças em grandes culturas como soja e trigo. No entanto, a alguns anos o controle biológico tem ganhado destaque. Assim, avaliou-se o antagonismo das bactérias *Pseudomonas fluorescens*, *Pantoea agglomerans* e *Bacillus* sp. sobre os fitopatógenos *Corynespora cassicola* e *Drechslera tritici-repentis*, isolados previamente de folhas de soja e trigo, respectivamente. O experimento foi realizado em condições controladas, no Laboratório de Fitobacteriologia da Faculdade de Agronomia e Medicina Veterinária (FAMV), Universidade de Passo Fundo (UPF), Rio Grande do Sul, Brasil. Os tratamentos foram: T1: *P. fluorescens* + patógeno; T2: *P. agglomerans* + patógeno; T3: *Bacillus* spp. + patógeno e T4: patógeno (controle). Em cada experimento (*C. cassicola* e *D. tritici-repentis*) se utilizou um delineamento inteiramente casualizado, com seis repetições cada. Os dados foram submetidos a uma análise de regressão linear, obtendo também a taxa de aumento diário (slope). O tempo final foi submetido a um ANOVA, e as médias comparadas pelo teste de Tukey ( $P < 0,05$ ). *P. fluorescens*, *P. agglomerans* e *Bacillus* sp. reduziram o crescimento micelial em 74 e 87 %, de *C. cassicola* e *D. tritici-repentis*, respectivamente. Embora este trabalho foi realizado em condições *in vitro*, pode servir como base para outros de controle biológico, especialmente com respeito ao manejo de doenças causadas por *C. cassicola* e *D. tritici-repentis*, em condições de campo.

**Palavras-chave:** *Pseudomonas fluorescens*, *Pantoea agglomerans*, *Bacillus* sp., Inibição do crescimento micelial.



## 1. Introduction

Soybean (*Glycine max* L Merrill) and wheat (*Triticale* sp., *Triticum aestivum* L, and *Triticum durum*) are important crops worldwide. While soybean has high levels of protein, lipids, isoflavones, and dietary fiber (Shaheen et al., 2016; Moloi et al., 2021), wheat, a source of carbohydrates and proteins, commonly used in baking and cooking, is one of the most consumed cereals in the world, being the basis of the diet of many cultures (Zhao et al., 2019). However, the cultivation of these prominent crops is affected by biotic factors, such as phytopathogenic fungi, which can decrease yield (Amorim et al., 2018).

Target spot (*Corynespora cassiicola* Berk. & Curt. C.T. Wei) and tan spot (*Drechslera tritici-repentis* Died. Shoem.) are significant foliar pathogens in soybean (Soares and Arias, 2020) and wheat (Laribi et al., 2022), respectively, causing significant damage in both crops. Chemical control is the most used method in the management of these diseases, normally using fungicides from the carboxamide (SDHI), strobilurin (QoI), and triazole (DMI) groups. However, a few years ago low fungicide efficiency was observed, also known as “control failure”.

There are reports in Brazil on the loss of insensitivity of *C. cassiicola* to benzimidazoles (carbendazim), and of *D. tritici-repentis* to strobilurins and triazoles (Avozani et al., 2014; Tonin et al., 2017). It is also known more extreme cases, as observed by Teramoto et al. (2017), who found isolates of *C. cassiicola* highly insensitive to the fungicide cyproconazole, demonstrating that a dose 100 times higher is required when compared to that used in isolates considered sensitive to this molecule. Recently, this behavior has been related to several mutations found both in *C. cassiicola* (Rondon and Lawrence, 2019; Zhu et al., 2020) and in *D. tritici-repentis* (Sautua and Carmona, 2021; Lammari et al., 2020), which confer resistance to DMI, SDHI, and QoI fungicides. All this makes us think that the way of handling diseases such as target spot and tan spot should be changed.

Using microorganisms considered biocontrollers may be an option in the range of possibilities for disease management. Traditionally, the genera *Pseudomonas*, *Bacillus*, and *Pantoea* have been considered effective in managing some phytopathogenic fungi in various regions of the world (Ludwig and Moura, 2007; Correa et al., 2010; Dutkiewicz et al., 2016; Vicentini et al., 2022). Several species of these genera have demonstrated the ability to produce antibiotics, mechanisms of competition for resources with the pathogen, or induction of plant resistance (Dutkiewicz et al., 2016; Dimkić et al., 2022). Using biocontrol agents reduces the need to spray synthetic fungicides on

crops, which is considered an environmentally healthier procedure (Dutkiewicz et al., 2016).

In this way, the *in vitro* study of the interaction between pathogenic organisms and possible biocontrollers are essential to change paradigms regarding the potential use of microorganisms on a large scale. Given these assumptions, the aim was to evaluate whether the bacteria *P. fluorencens*, *P. aglomerans*, and *Bacillus* sp. can reduce the mycelial growth and daily rate of progress of the pathogens *C. cassiicola* and *D. tritici-repentis*.

## 2. Material and Methods

The experiment was conducted under controlled conditions at the Phytobacteriology Laboratory of the Faculty of Agronomy and Veterinary Medicine (FAVM), University of Passo Fundo (UPF), Rio Grande do Sul, Brazil.

Monosporic isolates of *Corynespora cassiicola* and *Drechslera tritici-repentis* were used, isolated from lesions on leaf tissues of commercial soybean and wheat crops, respectively, belonging to the collection of the Laboratory of Mycology at UPF. Fungi reactivation was conducted in Petri dishes containing Potato Dextrose Agar (PDA) culture medium, incubating them for 13 days at 25 °C and a 12 h photoperiod. The bacteria *Pseudomonas fluorencens*, *Pantoea aglomerans*, and *Bacillus* sp. used in the present study as possible biocontrollers were obtained from the collection of the Laboratory of Phytobacteriology of the UPF.

Using the double-layer diffusion technique, an *in vitro* antagonism test was performed between the three bacteria and the two fungi. In Petri dishes containing PDA culture medium, an aliquot (100 mL) of a dilution (quantified in a spectrophotometer at a wavelength of 550 nm) of *P. fluorencens* (0.488 nm), *Bacillus* sp. (0.470 nm), and *P. aglomerans* (0.469 nm), obtained from 48 h old colonies. For the control, a saline solution was used. Subsequently, discs (6 mm Ø) of *C. cassiicola* and *D. tritici-repentis* were placed in the center of the Petri dishes. The daily growth of each fungus was evaluated, where it was measured every 24 hours, using a digital caliper, up to six days after the establishment of the experiment.

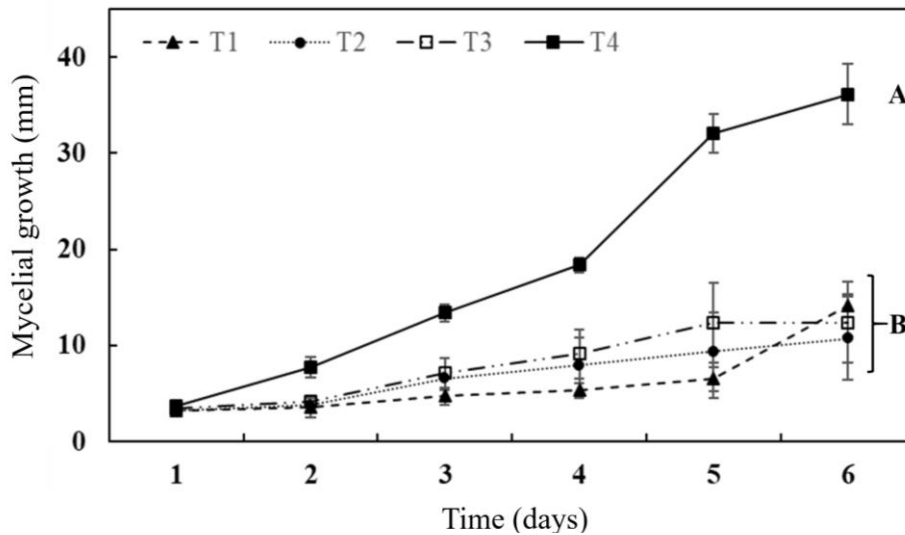
According to the pathogens, the treatments were: T1: *P. fluorencens* + pathogen; T2: *P. aglomerans* + pathogen; T3: *Bacillus* spp. + pathogen, and T4: pathogen (control – saline solution). In each experiment (*C. cassiicola* and *D. tritici-repentis*), a completely randomized design with six replications (Petri dishes) was used. The means obtained were analyzed using linear regression analysis, also obtaining the daily increase rate (slope). The data final times were

submitted to the ANOVA, after which these averages were compared using the Tukey test ( $P < 0.05$ ).

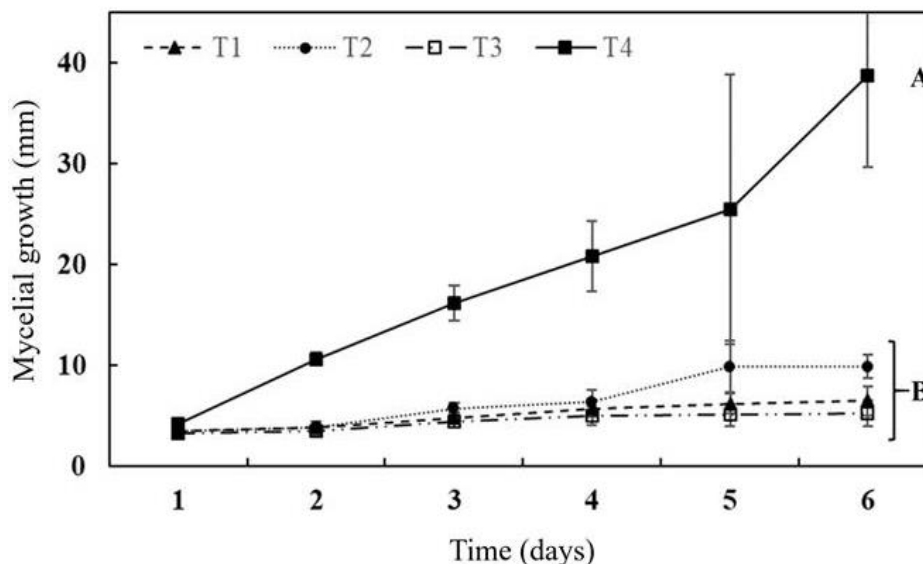
### 3. Results and Discussion

Mycelial growth of *C. cassicola* in Petri dishes containing culture medium and saline solution was different compared to treatments with *P. fluorescens*, *P. agglomerans*, and *Bacillus* sp. from the second day of the experiment (Figures 1 and 3). This behavior was more

pronounced at the end of the experiment, where *C. cassicola* grew significantly more in saline solution (36 mm), this mycelial growth being five times greater than that of the dishes containing the pathogen and bacteria. Mycelial growth of *D. tritici-repentis* was significantly higher when the pathogen was only in saline solution, compared to when exposed to bacteria from the second day of the experiment (Figures 2 and 3). The pathogen showed an average growth of 39 mm, 7.5 times greater than the dishes containing the pathogen and bacteria.



**Figure 1.** Mycelial growth (mm) of *Corynespora cassicola* in Petri dishes containing culture medium and *Pseudomonas fluorescens* (T1), *Pantoea agglomerans* (T2), *Bacillus* sp. (T3), and saline solution (T4, control), for six days. Passo Fundo, RS, Brazil. The bars represent the standard deviation of each treatment in the periods (in days) analyzed. Regression equations: T1:  $C = 1.2681d + 6$ ;  $R^2 = 0.92$ ; T2:  $C = 2.4257d + 6$ ;  $R^2 = 0.91$ ; T3:  $C = 3.131d + 6$ ;  $R^2 = 0.90$ ; T4:  $C = 9.8175d + 6$ ;  $R^2 = 0.87$ .



**Figure 2.** Mycelial growth (mm) of *Drechslera tritici-repentis* in Petri dishes containing culture medium and *Pseudomonas fluorescens* (T1), *Pantoea agglomerans* (T2), *Bacillus* sp. (T3), and saline solution (T4, control), for six days. Passo Fundo, RS, Brazil. The bars represent the standard deviation of each treatment in the periods (in days) analyzed. Regression equations: T1:  $C = 1.2161d + 6$ ;  $R^2 = 0.96$ ; T2:  $C = 2.1887d + 6$ ;  $R^2 = 0.86$ ; T3:  $C = 0.8202d + 6$ ;  $R^2 = 0.88$ ; T4:  $C = 9.9920d + 6$ ;  $R^2 = 0.91$ .

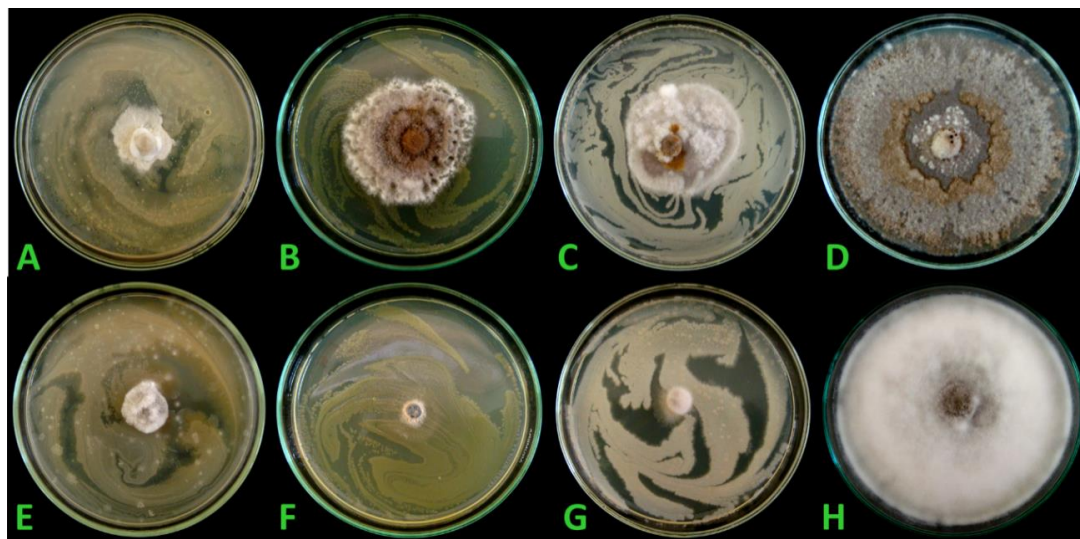
The regression analysis results were significant (Table 1). The mycelial growth rate of *D. tritici-repentis* and *C. cassiicola* was  $6.4 \pm 0.6$  and  $6.9 \pm 0.7$  mm day<sup>-1</sup>, respectively, when both pathogens were exposed only to saline solution. Thus, the inhibition of the area occupied by the pathogen mycelia was 74 and 87%, respectively, for *C. cassiicola* and *D. tritici-repentis* when exposed to biocontrol agents, *P. fluorencens*, *P. aglomerans*, and *Bacillus* sp.

The control of phytopathogenic fungi using bacteria of the genus *Pseudomonas* (Rodriguez and Pfender, 1997, Yang et al., 2014, Vicentini et al., 2022), *Pantoea* (Dutkiewicz et al., 2016), and *Bacillus* (Ferraz et al., 2008, Bach et al., 2016) has been reported in the scientific literature. According to Asaturova et al. (2022), when using *B. velezensis* against *D. tritici-repentis*, a reduction in the mycelial growth of pathogens of up to 94.3% was observed at 15 days in *in vitro* tests.

Inhibition of pathogens occurs at the cellular level, for instance, *D. tritici-repentis* mycelium phytopathogen associated with *Bacillus* would show shortening of cells, plasmolysis of conidia, changes in germ tubes,

vacuolation of hyphae, and formation of compounds in hyphae or the culture medium. This shortening of the suppressive effects of *Bacillus* concerning a wide list of phytopathogens can occur due to the effect of secondary metabolites of various chemical natures that these bacteria produce and promote protection through an antagonistic effect. The interaction of these bacteria with the plant promotes the release of lipopeptides in the physiology of plants, which play an essential role in inducing plant immunity, promoting protection by induced resistance (Larran et al., 2016, Asaturova et al., 2022).

Some species of the genus *Bacillus* also have the ability to inhibit the growth of pathogens and control of diseases, which would make it easier the availability of nutrients and/or the release of growth hormones in plants (Bach et al., 2016). These properties arouse interest in the use of biocontrol agents as well as the formulation of biofungicides. For example, according to Fernandes et al. (2021), in the United States, the use of *Bacillus* in commercial biofungicides began in 1983 for the treatment of peanut seeds.



**Figure 3.** Mycelial growth of *Corynespora cassiicola* (A-D) and *Drechslera tritici-repentis* (E-H) in Petri dishes containing culture medium and *Pseudomonas fluorencens* (A-E), *Pantoea aglomerans* (B-F), *Bacillus* sp. (C-G) and saline solution – control (D-H), at six days after the establishment of the experiments. Passo Fundo, RS, Brazil.

**Table 1.** Mycelial growth rate of *Corynespora cassiicola* and *Drechslera tritici-repentis* in Petri dishes containing culture medium, and *Pseudomonas fluorencens*, *Pantoea aglomerans*, *Bacillus* sp., and saline solution for six days. Passo Fundo, RS, Brazil.

Treatments	<i>Corynespora cassiicola</i>		<i>Drechslera tritici-repentis</i>	
	Rate (slope)	P-value	Rate (slope)	P-value
<i>Pseudomonas fluorencens</i>	$1.83 \pm 0.58$ b	0.0339	$0.65 \pm 0.10$ b	0.0002
<i>Pantoea aglomerans</i>	$1.59 \pm 0.12$ b	0.0002	$1.50 \pm 0.20$ b	0.0019
<i>Bacillus</i> sp.	$2.10 \pm 0.21$ b	0.0006	$0.40 \pm 0.10$ b	0.0063
Saline solution	$6.90 \pm 0.70$ a	0.0005	$6.40 \pm 0.60$ a	0.0006

Means followed by the same letter in the column do not differ according to the Tukey test ( $P < 0.05$ ).

In the study of Vasebi et al. (2015), they demonstrated that *P. agglomerans* inhibited 90% of the mycelial growth of *Macrophomia phaseolina*, a fungus that causes damage in soybean crops and other plant species. *P. agglomerans* has an inhibitory effect on the growth of numerous plant and animal pathogens, in addition to presenting other benefits for plant species *i.e.* promoting growth, assisting in obtaining nutrients, and promoting the induction of resistance to diseases and pests by activating metabolic pathways of plant physiology (Dutkiewicz et al., 2016). These characteristics are also present by some species of the genus *Bacillus* (Bach et al., 2016) and *Pseudomonas* (Vicentini et al., 2022).

In the present study, the bacteria used showed an inhibitory effect on the mycelial growth of the pathogens (Figures 1, 2, 3, Table 1). This can be explained by the competitive type of ecological interactions that these organisms perform with each other. Biocontrol can occur through direct or indirect mechanisms, mainly through antibiosis or physical interaction with the pathogen.

According to Vicentini et al. (2022), the direct inhibitory effect is given by the release of antifungal substances, such as protease, chitinase, and phosphatase that will act on the mycelia, causing the inhibition of their growth and/or development, in addition to causing injuries to the already formed cells. Another bacterial strategy to impair the mycelial growth of the pathogen is the production of a biofilm. This mechanism occurs when, bacterial colonies produce a layer of physical and biochemical protection that involves the cluster of cells, being able to also physically adhere to the mycelia (Ribeiro et al., 2016).

The formulation of commercial biofungicides has gained a substantial market (Meyer et al., 2016, Meyer et al., 2019, Agrofit, 2022). Its use *in vivo* can bring, in addition to controlling severity and incidence, other benefits for plant growth and yield (Bach et al., 2016, Dutkiewicz et al., 2016, Larran et al., 2016, Asaturova et al., 2022, Vicentini et al., 2022). Future studies are needed with these bacteria in formulations for *in vivo* tests to compare their social, environmental, economic, and agronomic impact, having as reference the chemical control of these pathogens.

#### 4. Conclusions

We have concluded that isolates of *Pseudomonas fluorescens*, *Pantoea agglomerans*, and *Bacillus* spp. could control the mycelial growth of the pathogens *Corynespora cassiicola* and *Drechslera tritici-repentis*, and thus have the potential for biocontrol of diseases related to these pathogens.

#### Authors' Contribution

Aveline Avozani, Andréia Tumelero, and Rosane Tonin: conceptualization, methodology and validation. Norimar Denardin: conceptualization, resources, supervision, and writing. Abimael Silva: data curation, original draft preparation, and editing. Felipe Garcés-Fiallos: conceptualization, resources, supervision, methodology, original draft preparation, and editing. All authors have read and agreed to the published version of the manuscript.

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