# Evaluation of the Antifungal Activity of *Streptomyces* sp. on *Bipolaris sorokiniana* and the Growth Promotion of Wheat Plants

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**Abstract**

*Streptomyces* sp. R18(6) and *Streptomyces* sp. 6(4) strains were evaluated for their ability to control brown spot and common root rot caused by *Bipolaris sorokiniana* in wheat crops. The antifungal activity of these isolates was tested using a double-layer assay and culture pairing at 28 °C. Physiological and enzymatic activity performed through siderophore, indole-3-acetic acid, nitrogen fixation and phosphate solubilization tests. The biocontrol of the disease and growth-promoting efficiency of wheat seedlings were assessed using *in vivo* assays in a greenhouse. In the culture pairing assays, both strains inhibited *B. sorokiniana* mycelial growth, while in the double-layer only *Streptomyces* sp. R18(6). *Streptomyces* sp. 6(4) produced auxin, siderophores, fixed nitrogen and solubilized phosphate, whereas R18(6) did not produce siderophores. In the greenhouse assays, strain R18(6) showed statistical differences in shoot dry mass and root dry mass compared with those of strain 6(4) in the presence of the phytopathogen (P ≤ 0.05), and these results were more evident when the environmental temperature was higher. In the absence of the phytopathogen, *Streptomyces* sp. 6(4) strain increased the root dry mass compared with that of the control during the same period. Therefore, these isolates can potentially control root rot and brown spotting and may promote the growth of wheat plants.

**Keywords:** Actinobacteria, *Bipolaris sorokiniana*, *Triticum aestivum* L., biocontrol brown spot, growth promoting

## 1. Introduction

Wheat is the second largest cultivated crop in the world (MAPA, 2016) mainly due to its presence in the diets of most nations (BNDES, 2016). As such, wheat is of vital importance to the global agricultural economy. The cultivation of this cereal is subject to diseases that compromise its production. Among these diseases are those caused by the phytopathogenic fungus *Bipolaris sorokiniana*.

The fungus *Cochliobolus sativus* is the teleomorph of *B. sorokiniana* (anamorph), which is the causal agent of brown spot, seed rot, and common root rot in wheat (Reis, 1988; Rashid et al., 2004; Duveiller et al., 2005). These diseases cause high losses in the production of this cereal (Reis & Casa, 2005). *Bipolaris sorokiniana* affects wheat and barley crops in various parts of the world, including Brazil, Canada, Australia, Europe, Asia and Africa (Diehl et al., 1982; Zillinsky, 1984; Stubbs et al., 1986; Reis, 1988; Tinline et al., 1988; Agrios, 1997).

The extensive use of fungicides to control diseases in crop plantations has resulted in resistant fungi, which has led to the accumulation of compounds potentially dangerous to both humans and the environment and as well increases in the costs of crop production. Biological control is an efficient and sustainable alternative for disease control in plants. Different non-pathogenic microbial species of *Bacillus* spp., *Pseudomonas* spp., *Trichoderma* spp., *Streptomyces* spp. and *Fusarium* spp. have been effectively used to control soil-borne phytopathogens (Paulitz & Belanger 2001; Haas & Keel, 2003; Jacobsen et al., 2004; Raza et al., 2013).

The phylum of Actinobacteria presents essential characteristics for biocontrol, including the ability to produce antifungal metabolites in the rhizosphere, promote plant growth, fix nitrogen, solubilize phosphates and produce siderophores and phytohormones (Patten & Glick, 2002; Tokala et al., 2002; Hamdali et al., 2008a; Chater et al., 2010). Active metabolites produced by species of *Streptomyces* compose approximately 60% of the products

used in agriculture (Ilic et al., 2007). Different studies have implicated various species of *Streptomyces* as biocontrol agents and plant growth promoters (Shrivastava et al., 2015; Sangdee et al., 2016; Shen et al., 2016; Toumatia et al., 2016).

The goal of this study was to evaluate the potential of two actinobacteria strains for controlling brown spot and common root rot in wheat plants and for promoting the growth of these plants under greenhouse conditions.

## 2. Materials and methods

### 2.1 Isolates

Fungal isolates *Bipolaris sorokiniana* 98004 (Cruz Alta - RS), 98012 (Lagoa Vermelha - RS) and 98032 (Engenheiro Beltrão - PR) were selected based on results obtained in previous pathogenicity tests (Minotto et al., 2014).

Two actinobacterial strains were isolated from the roots of healthy tomato plants by Oliveira et al. (2010). These isolates were recovered on plates containing starch casein agar (SCA: 10 g of starch, 0.3 g of casein, 2.0 g of $KNO_3$, 2.0 g of NaCl, 2 g of $K_2HPO_4$, 0.05 g of $MgSO_4 \cdot H_2O$, 0.02 g of $CaCO_3$, 0.01 g of $FeSO_4 \cdot 7H_2O$, 15 g of agar and distilled water up to 1L), incubated at 28 °C for 7 days. A partial 16S rDNA of the two strains were sequenced and deposited in the GenBank database under the accession numbers KY549728 (*Streptomyces* sp. strain R18(6)) and KY549799 (*Streptomyces* sp. strain 6(4)).

### 2.2 Production of Fungal Inoculum

*Bipolaris sorokiniana* strains were inoculated on plates containing carrot agar (200 g of crushed and ground carrot, 200 mL of distilled water and 4 g of agar) and incubated for 10 days at 28±2 °C under a photoperiod of 12 h (12 h light/12 h dark). After incubation, 5 mL of sterile saline solution (0.9%) containing Tween-20 surfactant (polyoxyethylene sorbitan monooleate) was added to the colonies and spread with a Drigalski loop. The final spore concentration was adjusted to $10^4$ spores/mL by counting conidia using a Neubauer chamber.

### 2.3 Antifungal Activity

The antifungal activity of the actinobacterial isolates was determined using plate diffusion method and a double-layer assay as well as by the pairing of cultures. Under aseptic conditions, the *Streptomyces* sp. strains R18(6), and 6(4) were spot-inoculated onto SCA (Starch casein agar) medium and incubated for 7 days at 28 °C. After this period, the antagonism between the actinobacteria and *B. sorokiniana* strains (98004, 98012 and 98032) was evaluated using the double-layer agar method. For this procedure, 10 mL of potato dextrose agar (PDA) overlay medium was inoculated with $10^4$ spores/mL fungal suspension. The plates were incubated for 4 days at 28 °C in the absence of light. The antifungal index was determined by the halo/colony ratio, obtained by the ratio of the mean of halo diameter by the mean of colony diameter (Rosato et al., 1981).

For the culture pairing assays, discs 5 mm in diameter containing *B. sorokiniana* (98004) were transferred to plates containing PDA medium at a distance of 1 cm from the edge of the dish. At the same time,  at the opposite side of the dish, the actinobacteria was also inoculated. Plates were then incubated at 28 °C for 10 days. The inhibition of fungal growth (%) was calculated using the formula (R1 – R2/R1) × 100, where R1 is the radial growth of inoculated fungi without exposure to actinobacteria and R2 is the radial growth of fungi inoculated with actinobacteria. The assay control consisted of a PDA plate inoculated with the *B. sorokiniana* and a plate for each actinobacteria strain, in the same manner as described before. Culture growth was observed every day until it covered the entire surface of the plate. The experiment was carried out in triplicate.

### 2.4 Phosphate Solubilization

Phosphate solubilization assays were performed following the protocol of Nautiyal (1999). Plates containing NBRIP medium were inoculated with the actinobacterial isolates and incubated at 28 °C for 21 days. The assay was performed in triplicate, and the evaluation determined by the presence or absence of halos under colony growth.

### 2.5 Siderophore Production

Siderophore assays were performed by the method proposed by Schwyn and Neilands (1987) modified by Silva-Stenico et al. (2005). *B. sorokiniana* strains were inoculated onto King B medium adapted by Glickmann and Dessaux (1995) (1 g of peptone, 0.0575 g of $K_2HPO_4$, 0.075 g of $MgSO_4$, 0.75 g of glycerol and 250 mL of distilled water [pH 6.8]). One milliliter aliquots were withdrawn every 48 h, placed in microcentrifuge tubes and centrifuged at 13,000 rpm for 5 min. Afterward, 500 μL of the supernatant was transferred to new tubes containing 500 μL of chromoazurol-S (CAS) dye (To prepare the CAS dye, 60.5 mg of CAS in 50 mL of distilled water was added to 10 mL of $FeCl_3$ solution; the solution was then stirred, and a solution of 72.9 mg of

hexadecyltrimethylammonium bromide [CTAB] previously dissolved in 40 mL of water was slowly added, after which the solution was autoclaved for 15 min.). A positive reaction was indicated by the change in color from blue to orange or yellow during a period of 15-30 min.

### 2.6 Auxin Production

Auxin evaluation was carried out by the method of Gordon and Weber (1951). The isolates were previously grown in 10% tryptic soy broth medium supplemented with 5 mM tryptophan and incubated at 28 °C under agitation at 115 rpm. Every 48 h for 264 h, 2 mL of the culture was transferred to a microcentrifuge tube and centrifuged at 13,000 rpm for 5 min. Auxin production was determined by transferring 500 µL of the supernatant to tubes containing 500 µL of Salkowski reagent (2.4 g of $FeCl_3$ and 84.2 mL of $H_2SO_4$). The tubes were stored in the dark at room temperature for 30 min, and the color intensity was determined spectrophotometrically at $\lambda = 520$ nm. A calibration curve was prepared using different concentrations of auxin (0.2, 5.625, 11.25, 22.55 and 45 µg/mL).

### 2.7 Nitrogen Fixation

The two actinobacterial strains were cultured in cotton-capped penicillin tubes containing 10 mL of NFb medium (Döbereiner et al., 1995) incubated at 28 °C and evaluated after 14 days.

### 2.8. Seed Infestation

Seeds were subjected to surface disinfestation by immersion in 70% ethanol (2 min) and 2.5% sodium hypochlorite (2 min) followed by three consecutive washes with sterile distilled water. The fungal strains were multiplied in casein starch broth at 28±2 °C under agitation at 115 rpm for 72 h. After this period, 5 mL of the suspension was transferred to new flasks containing 50 mL of the same culture medium and then incubated under the same conditions as above. Afterward, the concentration of propagules was $10^8$ colony forming units (CFU)/mL. Seed infestation was performed by depositing 25 seeds in this suspension and maintaining it under stirring for 4 h at 25 °C.

### 2.9 In vitro Colonization of Wheat Seedlings by Actinobacteria Strains R18(6) and 6(4)

To evaluate the ability of the isolates to colonize the wheat plantlet root system, we followed the protocol described by Queiroz et al. (2006), with modifications. The seeds of wheat cultivars Tbio Mestre and Marfim were disinfested, dried on sterile filter paper and infested with the actinobacteria strains. Afterward, one of the seeds was transferred to test tubes containing agar-water culture medium (0.6%). The tubes were incubated for germination at 25 °C for 7 days under 12-h photoperiod. The evaluation was carried out by observing the tubes for medium turbidity around the root system, which indicates the presence of the bacterial growth. The presence of microorganisms colonizing the surface and internal tissues of the roots were observed by cutting the roots into small fragments (2-3 cm) and deposited in Petri dishes containing SCA culture medium. The experimental design of this assay was completely randomized, involving the two actinobacteria and five replicates. The control consisted of disinfected seeds immersed in saline solution.

### 2.10 Greenhouse Assay

The experiment was performed in a greenhouse of the Department of Phytosanitary at Faculty of Agronomy/Universidade Federal do Rio Grande do Sul-UFRGS, Porto Alegre, RS, Brazil in April to June and June to August of 2016. The assays were performed in disposable plastic cups (500 mL) with Green Plus® substrate composed of soil and expanded vermiculite (1:1). In each pot, five wheat seeds of the cultivar TBio Mestre were sown following the description each treatment. After the germination of the seeds, thinning was performed, leaving two seedlings per pot. The experiments were maintained for nine weeks.

A *B. sorokiniana* suspension of $10^4$ spores/mL of isolate 98004 was prepared, and infestation was applied in two ways: 1) substrate inoculation using the fungal suspension at the sowing groove or 2) spraying of the suspension onto the aerial portion of the plant with an atomizer (air compressor model AS 176 [40 psi] and Steula BC64 pistol) at a distance of 40 cm from the leaves.

To accomplish this study, seven treatments were designed for each actinobacterium isolate: treatment 1 (growth promoter), seeds microbiolized with actinobacteria isolate; treatment 2, seeds microbiolized with actinobacteria/*B. sorokiniana* inoculated in the substrate; treatment 3, seeds microbiolized with actinobacteria/*B. sorokiniana* infestation by aerial spraying; treatment 4, seeds microbiolized with actinobacteria suspension at planting/*B. sorokiniana* infestation by aerial spraying; treatment 5 (control), seeds without actinobacteria; treatment 6 (control), seeds infested with *B. sorokiniana* at planting; and treatment 7 (control), seeds without microbiolization of actinobacteria/*B. sorokiniana* infestation by aerial spraying.

In treatment 2, the substrate was inoculated with a suspension ($10^4$ spores/mL) of *B. sorokiniana* at the GS 15 and 35 stages (Zadoks et al., 1974). In treatment 4, a suspension ($10^8$ CFU/mL) of the antagonist was applied to the seeds in the sowing groove. In treatments 3, 4 and 7, a suspension of *B. sorokiniana* was sprayed on wheat plants at the GS 15 and 35 stages. The plants were placed in a humidity chamber for 24 h before spraying and for 48 h after spraying. At the end of the experiment, the shoot height, root size, fresh weight and dry weight of wheat plants were determined.

### 2.11 Evaluation of the Severity of Brown Spot on Wheat Leaves

Forty-eight hours after spraying, the plants were maintained in the greenhouse without temperature control and under natural lighting conditions. One vessel was randomly selected from each treatment that received the *B. sorokiniana* suspension for evaluation of the disease. From these plants, the fourth or fifth leaf was located and fixed with adhesive tape onto a flat surface together with a 20 cm ruler for software calibration. These leaves were photographed with a Sony Alpha 35 digital camera at a distance of 20 cm. The period of observation was every two days until the $10^{th}$ day. The leaf area was performed by ImageJ software (http://rsbweb.nih.gov/ij/). The program determined the total area, healthy and injured area (necrotic and chlorotic) of the leaves. The data were stored in Excel® software to calculate the percentage of the injured area concerning the total area evaluated.

### 2.12 Statistical Analysis

The greenhouse test was conducted in five replicates. The analysis of variance was performed, and the means were compared by the Bonferroni test, both at $P \leq 0.05$, using the software program SPSS version 18.

## 3. Results

### 3.1 Antifungal Activity

The results of the double-layer assay showed that only strain *Streptomyces* sp. R18(6) could inhibit the growth of the three *B. sorokiniana* (98004, 98012 and 98032) strains tested (Figure 1). Strain 6(4) did not show activity in the double-layer assay against *B. sorokiniana* isolates. The best antibiosis index of R18(6) was observed against *B. sorokiniana* 98032 with a diameter of 3.82 cm.
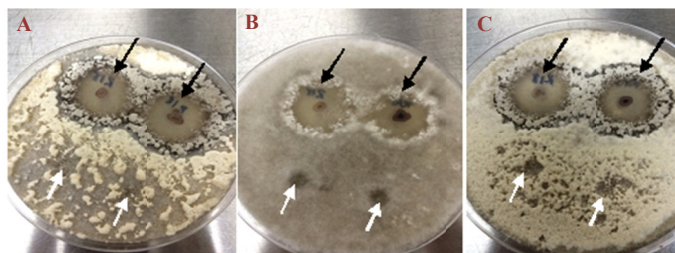


Figure 1. Inhibition halos formed by isolate R18(6) against three *B. sorokiniana* isolates in the double-layer assay. (A) *B. sorokiniana* 98004; (B) *B. sorokiniana* 98012; (C) *B. sorokiniana* 98032. Black arrow indicates the inhibition of isolate R18(6). White arrow indicates the growth of isolate 6(4)

In the culture pairing assay, the two actinobacterial strains inhibited the radial growth of *B. sorokiniana* isolate 98004 (Figure 2). The percentage of radial growth inhibition of *B. sorokiniana* isolates was calculated: strain R18(6) inhibited 33.7% of the *B. sorokiniana* radial growth and strain 6(4), 44.6%.
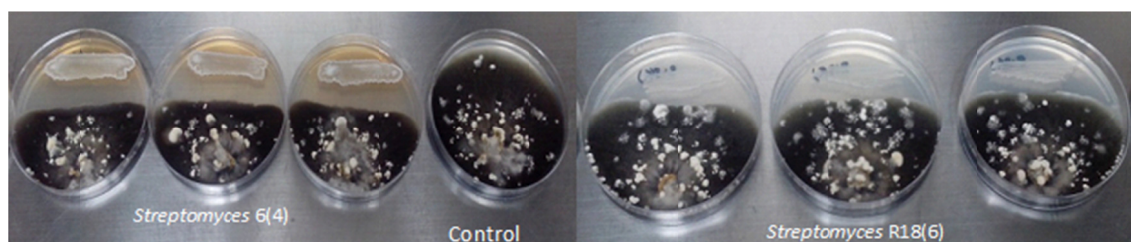


Figure 2. Inhibition of *B. sorokiniana* 98004 radial growth on PDA medium. Pairing of culture of the isolates of actinobacteria 6(4) are to the left of the control and R18(6) to the right of the control

### 3.2 Phenotypic Tests

*Streptomyces* sp. R18(6) and 6(4) were able to solubilize phosphate, fix nitrogen and produce siderophores. For strain R18(6), siderophore production was not observed with the method used in this work.

*Streptomyces* sp R18(6) showed a maximum auxin production of 6.98 μg/mL after 264 h of growth while strain 6(4) showed a maximum auxin production of 10.75 μg/mL after 192 h of growth (Figure 3).
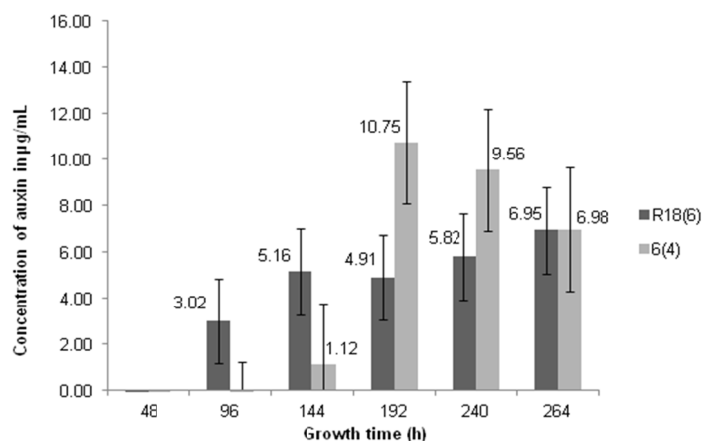


Figure 3. Auxin concentrations (μg/mL) produced by *Streptomyces* isolates R18(6) and 6(4) after 264 h of incubation at 28 °C

The roots of wheat seedlings of the two cultivars were microbiolized with the two actinobacterial strains. Medium turbidity along roots is a characteristic of bacterial growth. Confirmation of the actinobacteria colonization of the rhizosphere was obtained by the growth of isolates in SCA.

In the experiment carried out in the greenhouse during April to June, statistical differences were observed between the actinobacterial strains (treatment 2: seeds infested with actinobacteria and inoculation of the phytopathogen in the substrate) for root dry mass and shoot dry mass. Strain R18(6) showed the best results with 560 mg of shoot dry mass and 250 mg of root dry mass compared to the results for isolate 6(4) (330 mg and 100 mg, respectively) (Tables 1 and 2).

Table 1. Average aerial portion dry weight and height for each treatment during the periods of April-June and June-August

| Treatment | April-June | | | | June-August | | | |
|---|---|---|---|---|---|---|---|---|
| | Height (cm) | | Aerial dry weight (mg) | | Height (cm) | | Aerial dry weight (mg) | |
| | 6(4) | R18(6) | 6(4) | R18(6) | 6(4) | R18(6) | 6(4) | R18(6) |
| 1 | 49.1 [a] | 45.2 [ab] | 540 [a] | 480 [a] | 49.4 [a] | 50.9 [a] | 740 [a] | 670 [a] |
| 2 | 44.5 [ab] | 48.6 [a] | 330 [b] | *560 [a] | 48.3 [a] | 49.2 [a] | 670 [a] | 550 [a] |
| 3 | 42.8 [bc] | 43.0 [bc] | 260 [b] | 260 [b] | 40.3 [c] | 36.9 [c] | 330 [b] | 440 [b] |
| 4 | 42.8 [bc] | 43.7 [bc] | 350 [b] | 310 [b] | 39.5 [c] | 40.4 [c] | 410 [b] | 390 [b] |
| 5 | 46.5 [ab] | | 470 [a] | | 48.9 [a] | | 710 [a] | |
| 6 | 41.3 [c] | | 220 [b] | | 44.0 [b] | | 390 [b] | |
| 7 | 37.5 [d] | | 250 [b] | | 37.7 [c] | | 330 [b] | |

*Note*. (1) Seeds infested with R18(6) or 6(4); (2) seeds infested with R18(6) or 6(4)/*B. sorokiniana* in the substrate; (3) seeds infested with R18(6) or 6(4)/*B. sorokiniana* infested by aerial spraying; (4) seeds infested with R18(6) or 6(4) suspension at planting/*B. sorokiniana* infested by aerial spraying; (5) seeds without infestation (control); (6) seeds infested with *B. sorokiniana* (control); (7) seeds without infestation/spraying of *B. sorokiniana* (control). * Significant statistical difference between the bacteria by the Bonferroni test ($P \leq 0.05$). Means followed by same letters in the column do not differ statistically from each other by the Bonferroni test ($P \leq 0.05$).

Table 2. Average dry weight and root length for each treatment during the periods of April-June and June-August

| Treatment | April-June | | | | June-August | | | |
|---|---|---|---|---|---|---|---|---|
| | Dry root weight (mg) | | Root lenght (cm) | | Dry root weight (mg) | | Root lenght (cm) | |
| | 6(4) | R18(6) | 6(4) | R18(6) | 6(4) | R18(6) | 6(4) | R18(6) |
| 1 | 370 [a] | 320 [a] | 21.9 [ab] | 22.4 [ab] | 120 [a] | 160 [a] | 27 [a] | 35.2 [a] |
| 2 | 100 [c] | *250 [b] | 19.2 [bc] | 20.9 [bc] | 90 [b] | *120 [a] | 30.8 [a] | 28.1 [a] |
| 3 | 90 [c] | 80 [c] | 19.9 [bc] | 18.3 [bc] | 60 [c] | 60 [c] | 27.7 [a] | 25.2 [a] |
| 4 | 90 [c] | 100 [c] | 27.9 [a] | 20.4 [bc] | 60 [c] | 70 [c] | 28.6 [a] | 30.1 [a] |
| 5 | 250 [b] | | 25.5 [a] | | 140 [a] | | 25.9 [a] | |
| 6 | 110 [c] | | 16.17 [c] | | 90 [b] | | 29.2 [a] | |
| 7 | 90 [c] | | 21.1 [bc] | | 50 [c] | | 27.6 [a] | |

*Note*. (1) Seeds infested with R18(6) or (4); (2) seeds infested with R18(6) or 6(4)/*B. sorokiniana* in the substrate; (3) seeds infested with R18(6) or 6(4)/*B. sorokiniana* infested by aerial spraying; (4) seeds infested with R18(6) or 6(4) suspension at planting/*B. sorokiniana* infested by aerial spraying; (5) seeds without infestation (control); (6) seeds infested with *B. sorokiniana* (control); (7) seeds without infestation/spraying of *B. sorokiniana* (control). * Significant statistical difference between the bacteria by the Bonferroni test ($P \leq 0.05$). Means followed by same letters in the column do not differ statistically from each other by the Bonferroni test ($P \leq 0.05$).

In treatment 1 (absence of phytopathogen), there were statistical differences between *Streptomyces* strain 6(4) and R18(6) compared with the control ($P \leq 0.05$) regarding root dry mass (Table 2) and shoot height (Table 1), indicating growth promotion.

Plants infested by spraying the phytopathogen onto the shoot (treatments 3, 4 and 7) were submitted to ImageJ software and the injured of leaf area estimated. With the results, it was possible to observe that in the treatments where the suspension of *Streptomyces* sp. 6 (4) was applied in the sowing groove the best result was obtained with the smallest area injured by the phytopathogen (Table 3, Figure 4). The treatment without application of the antagonist showed a greater severity of the disease in the leaves, comprising from 31.9% to 41.43% of the injured area (Table 3). These results indicate that the application of the antagonist to the sowing groove interferes with the resistance and protection of the plant against the phytopathogen *B. sorokiniana* (Figure 5).

Table 3. Porcentage of injure leaf área with *Bipolaris sorokiana* after inoculation with the *Streptomyces* sp. atrain R18(6) and 6(4)

| Tratamentos | % of injure leaf area | | |
|---|---|---|---|
| | 1ª evaluation | 2ª evaluation | 3ª evaluation |
| Microbiolized wheat seed *Streptomyces* strain 6(4) | 11.14 | 11.63 | 19.61 |
| Microbiolized wheat seed *Streptomyces* strain R18(6) | 14.31 | 29.10 | 43.79 |
| Inoculation of *Streptomyces* strain 6(4) in the sowing groove | 5.60 | 6.00 | 9.69 |
| Inoculation of *Streptomyces* strain R18(6) in the sowing groove | 9.01 | 17.16 | 27.80 |
| Absence of antagonist microorganism | 31.91 | 38.73 | 41.43 |
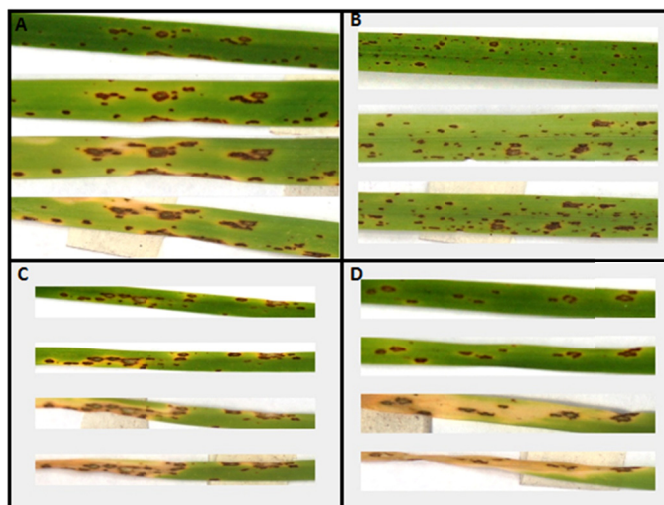
Figure 4. Responses of wheat plants to infestation of *Streptomyces* strains in the seed and *B. sorokiniana* spraying on the shoot. (A): seed microbiolized with *Streptomyces* sp. strain R18(6) suspension in the seeding groove and infestation of the shoot with *B. sorokiniana*; (B): seed microbiolized with *Streptomyces* sp. strain 6(4) suspension in the seeding groove and infestation of the shoot with *B. sorokiniana*; (C) and (D): seeds not microbiolized with *Streptomyces* strains or infestation of the shoot with *B. sorokiniana*
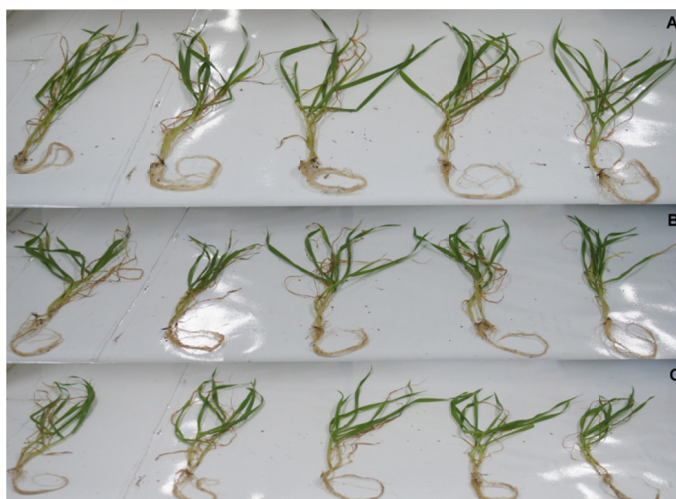


Figure 5. Responses of wheat plants root length to infestation of *Streptomyces* strains in the seed and *B. sorokiniana* spraying on the shoot. (A): seed microbiolized with *Streptomyces* sp. strain R18(6) suspension in the seeding groove and infestation of the shoot with *B. sorokiniana*; (B): seed microbiolized with *Streptomyces* sp. strain 6(4) suspension in the seeding groove and infestation of the shoot with *B. sorokiniana*; (C): seeds not microbiolized with *Streptomyces* strains or infestation of the shoot with *B. sorokiniana*

## 4. Discussion

Actinobacteria are quantitatively and qualitatively important in the rhizosphere, where they can improve plant growth and protect roots against invasion by root pathogens (Sardi et al., 1992; Crawford et al., 1993; Atalan et al., 2000; Basil et al., 2004; Palaniyandi et al., 2013; Sangdee et al., 2016).

In our experiment, it was possible to observe the *in vitro* antifungal activity of *Streptomyces* sp. strains *R18*(6) and 6(4). Although strain 6(4) showed antifungal activity only in the direct comparison of cultures, this isolate could inhibit *B. sorokiniana* 98004 growth by 44%. This observation may be a result of the stress and competition of the isolate due to the presence of another microorganism in the same environment or because they are growing in a culture medium (PDA) that does not completely favor its development; thus, there is increased expression of

metabolites.

Lu et al. (2016) reported in their experiment that 24% of actinobacteria isolates inhibited the mycelial growth of *Fusarium oxysporum* f. sp. *cucumerium* in PDA medium and that 84% of these isolates belonged to the genus *Streptomyces*. Taechowisan et al. (2003) performed a direct comparison of endophytic actinomycetes cultures with antifungal activity and reported that most of the isolates also belong to the genus *Streptomyces*.

Root colonization and auxin production by the R18(6) and 6(4) strains may have influenced the increase in root dry mass in treatment 1, treatment 5 and the control in the assay done in during April to June (Table 2).

Various *Streptomyces* species, such as *Streptomyces olivaceoviridi*, *Streptomyces rimosus* and *Streptomyces rochei*, isolated from the tomato rhizosphere can produce EIA and improve plant growth by increasing germination, root elongation and root dry weight (El-Tarabily, 2008).

Strain R18(6) influenced the increase of aerial dry mass of wheat plants, even in the presence of the phytopathogen (Treatment 2, Table 1-assay April-June). According to studies by Jog et al. (2014), Gopalakrishnan et al. (2013, 2014) and Palaniyandi et al. (2014), the inoculation of *Streptomyces* isolates with crops such as rice, wheat, sorghum and tomato increases the biomass of these plants. The actinobacterial isolate also exhibited other properties associated with biological control agents and plant growth, such as the ability to solubilize phosphates and to produce siderophores. Phosphate deficiency is a limiting factor in agricultural production; therefore, phosphorus solubilization and mineralization by bacteria are important features of plant growth-promoting bacteria (PGPB) (Richardson, 2001; Hamdali et al., 2008b). Actinobacteria such as *Streptomyces* and *Micromonospora* have been reported to be phosphate solubilizers (Hamdali et al., 2008a). Hamdali et al. (2008a) reported that the actinobacterial strain *Micromonospora aurantiaca* promotes plant growth and fitness in soil supplemented with rock phosphate.

In this sense, the microorganisms selected in the present study may play a beneficial role in plant development, since growth-promoting effects are also associated with the production of IAA (Khamna et al., 2009) and phosphate solubilization (Hamdali et al., 2008b). Despite its importance, phosphate solubilization has been reported in a small number of microorganisms (Hameeda et al., 2008). The production of siderophores by PGPB may limit iron uptake by the pathogen decreasing its proliferation capacity (Kloepper et al., 1980; Dowling et al., 1996). Schippers et al. (1987) suggested that this mechanism is effective because the PGPB produce siderophores have much greater affinity for available iron than do fungal pathogens.

PGPB can trigger a plant-based phenomenon known as systemic induced resistance, which is similar to systemic acquired resistance and occurs when plants activate their defense mechanisms in response to infection by a pathogen (Pieterse et al., 2009). Inoculation of the antagonist in the period before inoculation of the fungus in the soil may have induced systemic plant resistance, resulting in a greater tolerance to the phytopathogen, as observed in treatment 2 in the first assay. Moura et al. (1998) verified in their experiment that inoculating actinobacteria in tomato seeds before the phytopathogen improved the defense of the plant against *Ralstonia solanacearum*. Van Loon (2007) stated that rhizobacteria could reduce the activity of pathogenic microorganisms not only through antagonism but also by the activation of the plant to better defend itself.

The actinobacterial isolates used in this work showed antagonistic action against *B. sorokiniana*, preventing root rot in a greenhouse experiment and promoting the growth of wheat plants. Therefore, it can be suggested that these *Streptomyces* isolates can potentially be used as biocontrol agents for diseases caused by *B. sorokiniana*. Assays using a mixture of the two actinobacterial isolates and field tests to confirm the results obtained are suggested for future work.

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**Appendix**
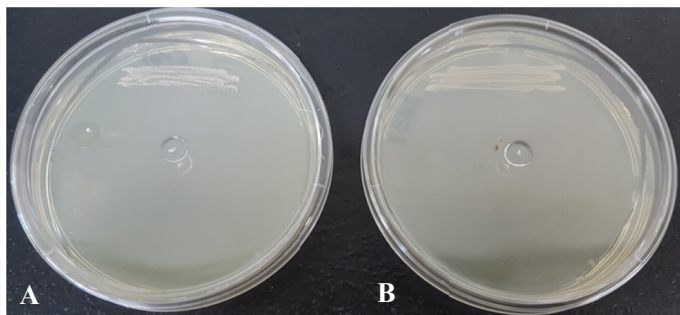


Appendix 1. A: Strain *Streptomyces* sp. 6(4) grown alone in PDA medium; B: Strain *Streptomyces* sp. R18(6) grown alone in PDA medium

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