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PHOSPHATE SOLUBILIZATION, INDOLE ACETIC ACID SYNTHESIS AND BIOCONTROL BY *STREPTOMYCES* ISOLATES

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ABSTRACT

Actinobacteria of the genus *Streptomyces* have great potential in producing several bioactive compounds. With that, the objective was to characterize morphologically isolates of *Streptomyces*, to evaluate the phosphate solubilization and the production of indole acetic acid (IAA) and the antagonistic activity to the pathogenic fungi *Curvularialunata* and*Colletotrichum* sp. The morphological characterization presented eight-gram positive isolates from the nine evaluated. Six isolates presented a colony greater than 2 mm, seven with elevated colony and five transparent. All isolates presented positive results to produce filamentous and branched hyphae, and only one isolate (UFT-St03) did not present mycelium staining. As for the phosphate solubilization capacity test, only one UFT-St07 isolate presented an index of phosphate solubilization. To produceAIA, the best results were superior in the presence of the L-tryptophan inducer, the best observed for UFT-St04 (3.8 μgmL^{-1}) and UFT-St08 (3.09 μgmL^{-1}). For the antagonism evaluation, the UFT-St05 and UFT-St07 isolates presented percentages of inhibition against the *C. lunata* pathogen of 90 and 93.7% inhibition, respectively. As for the *Colletotrichum* sp.pathogen, higher percentages of inhibition of the isolates UFT-St04 (85.2%) and UFT-St07 (87.6%) were observed.

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INTRODUCTION

The rhizosphere is the contact zone between soil and roots, having a great diversity of organisms, where bacteria, fungi, algae are found, among others. Rhizobacteria can solubilize phosphate and to produce metabolites directly related to plant growth, such as auxins, gibberellins and cytokinins. In addition, they synthesize antibiotics, siderophores and hydrocinnamic acid that reduce the pathogens activity (GRAÇAS *et al*., 2015). The solubilization of phosphate by microorganisms is important, since natural phosphates have the drawback of having low solubility and, therefore, are poorly available to plants. Among the phosphate solubilizing microorganisms, bacteria are found in greater numbers, and the solubilization of insoluble sources of phosphate may occur due to the excretion of organic acids by the microorganisms, which solubilize those phosphates by the environment acidification (MASSENSSINI *et al*., 2015). The production of indole acetic acid (IAA) has also received a lot of attention due to its importance, since it is a hormone directly linked to the regulation of plant growth. The main inducer of the IAA production route is the tryptophan amino acid (Trp). However, the biosynthesis of this hormone can also occur independently of the precursor Trp (SILVA & LANDGRAF, 2017). The IAA can be synthesized and excreted by several endophytic microorganisms, which can be cited the fungus *Trichoderma* sp. (OLIVEIRA *et al*., 2012) and the bacteria *Azospirillum brasiliensis* and *A. lipoferum* (KUSS *et al*., 2007), *Pseudomonas fluorescens* (HERNANDEZ *et al*., 2008) and *Bacillus* species (RATÓN *et al*., 2011). The effect of pathogenic species on agriculture is widely reported, once the losses arising from the attack of plant diseases in Brazil are around 10-20%.

Some of the diseases transmitted are white mold from beans and soybeans, fusariosis from many host species, and rot, among others (GOULART, 2014). Among the pathogenic species that cause great damage to agriculture can be cited the *Colletotrichum* genus, which causes anthracnose in numerous species of economic interest (ESPAÑA *et al*., 2017). In addition to these, other pathogens of secondary importance are detected quite frequently, causing damage to plants, such as the case of *Curvularia lunata* (GAO *et al*., 2017). Regarding the beneficial microorganisms found in the soil, it should be noted the importance of the bacterium *Streptomyces*, which is a genus that contains more than 600 species with published names (SHE *et al*., 2016). Members of the genus *Streptomyces* represent one of the largest phyla in the soil microbiota across the globe. They are aerobic actinomycetes, spore-forming and gram positive. The genomes of streptomyces carry many clusters of genes responsible to produce bioactive metabolites, such as enzymes, toxins, antibiotics, antitumor and antifungal agents (SHE *et al*., 2016). Thus, *Streptomyces* are potential bacteria in the production of metabolites with important application in agriculture. Thus, the present study aimed to characterize morphologically isolates of *Streptomyces* spp.,evaluate the capacity of phosphate solubilization and the production of indole acetic acid (IAA) and antagonism to the pathogenic fungi *Curvularialunata* and *Colletotrichum* sp.

MATERIAL AND METHODS

Phenotypic characterization of isolates: The experiments were conducted at the Microbiology Laboratory of the Federal University of Tocantins – *Campus* of Gurupi (UFT/PPGPV). *Streptomyces* spp. inoculum sources were isolated from different soil samples from regions of the state of Tocantins and were subsequently identified by means of macro and micro-morphological tests (WILLIAMS *et al*., 1989). Nine isolates were obtained, and the phenotypic characteristics evaluated were: growth time (1 to 8 days); colony diameter, shape, elevation, and transparency; production of filamentous and branched hyphae and mycelium staining; modification of the pH of the medium (HUNGRIA *et al*., 2001) and Gram stain test (KONEMAN, 2008).

Phosphate solubilization: For the phosphate solubilization test *in vitro*, the isolates of *Streptomyces* spp. were cultivated and replicated in modified PDA culture medium (Potato 20%, dextrose 2%, agar 1.5% and starch 1%; distilled water; pH 6.8), where they were incubated at 29 ºC for seven days. Plate Count Agar - PCA medium (Peptone 0.5%, Yeast extract 0.25%, glucose 0.1%, agar 15%, distilled water) was used, to which was added a solution containing 0.25 g $L^{-1}$of K_2HPO_4 and another containing 1 g $L^{-1}$of $CaCl_2$, for the formation of precipitated calcium phosphate. The bromothymol blue dye was added to the P-Ca medium, which aimed to visualize pH change of the medium by the bacteria, due to the change in the color of the medium, adjusting the pH to 6.6 to form precipitated calcium phosphate (HARA & OLIVEIRA, 2004). From the isolates previously incubated, disks of approximately 10 mm were made from the isolates in each Petri dish. Incubation was performed in BOD at 28 ± 1° C. The experiment was carried out in a completely randomized design with 6 replications. The evaluations were made at 48, 96, 144, 192, 240 and 288 hours after inoculation of the *Streptomyces* spp. isolates. Being evaluated for a period of 12 days. The diameter measurements (Φ) of the solubilization halos, perceived as a translucent area around the colony, and those of the Φ of the colonies were measured using a digital caliper. From these measurements, the solubilization indices of each isolate were obtained using the formula: SI = Φ Halo (mm) / Φ Colony (mm) (HARA; OLIVEIRA, 2004). From the SI, *Streptomyces* spp. were classified as strains with low (SI <2), medium (2 ≤ SI 4) and high solubilization (SI > 4) (HARA; OLIVEIRA, 2005).

Production of indole acetic acid: For the *in vitro* IAA production assay by the *Streptomyces* spp.Isolates,initially the isolates were previously cultivated in a Petri dish in a modified PDA culture medium (Potato 20%, dextrose 2%, agar 1.5% and starch 1%; distilled water; pH 6.8), where they were incubated in BOD at 29 ± 1 °C for eight days. The modified PD medium (Potato 20%, dextrose 2% and starch 1%; distilled water; pH 6.0) was used. The isolates were transferred through dishes of approximately 2-3 mm in diameter containing bacterial cell mass to Erlenmeyer flasks (250 mL) containing 100 mL with BD media in the absence (control) and presence of L-tryptophan. The concentration of L-tryptophan used was 100 mg L^{-1}. The experiment was carried out in a completely randomized design with four replications. The evaluations were made at 48, 96, 144 and 192 hours after inoculation of the *Streptomyces* spp. isolates. Being cultivated on a shaking table with orbital movement (Novatécnica®, 100 rpm at 26 ± 2 ºC). Subsequently, the bacterial cell biomass was separated by centrifugation (Excelsa®4 model 280R, 12,000 rpm for 15 min.). For the IAA colorimetric analysis, part of Salkowski's reagent [FeCl3 0.5 $molL^{-1}$ +$HClO_4$ (35%)] and two parts of the supernatant obtained from each isolate were used (GORDON; WEBER, 1951). After qualitative evidence of the presence of IAA (pink color after 25 minutes of reaction at a temperature of 26 ± 2 ºC in the dark), the phytohormone was quantified in a spectrophotometer at 530 nm. The concentrations, in μgmL^{-1}, were calculated from a standard curve with known concentrations of the synthetic form of the hormone (0 to 100 µg mL^{-1}), whose readings were the basis for calculating the concentration of IAA in the samples.

Antagonistic activity: The antagonistic activity of the nine isolates of *Streptomyces* spp. was tested against the pathogenic fungi *Curvularialunata* and *Colletotrichum* sp. The fungus *C.lunata* used was isolated from corn plants from the UFT experimental fieldwith symptoms of the disease. The phytopathogenic fungus *C.lunata* was characterized molecularly at the Integrated Plant Management Laboratory at UFT. The fungus *Colletotrichum* sp. alsoobtained from the collection of the Laboratory of Microbiology at UFT, was isolated from soybean plants with symptoms of anthracnose. The nine isolates of *Streptomyces* spp.were initially cultivated in a Petri dish in modified PDA culture medium (Potato 20%, dextrose 2%, agar 1.5% and starch 1%; distilled water; pH 6.8) and incubated in BOD at 29 ± 1 °C for seven days. The phytopathogenic fungus was also cultivated in a modified PDA medium and incubated for seven days.

The experiment was carried out in a completely randomized design with 4 replications. For the evaluation of antagonism, the fungal culture technique on antagonist culture was used. After seven days of growth of the *Streptomyces* spp. isolates, the inoculantswere plated in PDA medium with the help of a platinum loop. Then, dishes of approximately 6 mm containing culture medium with colonies of the phytopathogenic fungus with seven days of growth wereplaced in the center of the plates. Evaluations of the potential for antagonism were made at 48, 96, 144 and 192 hours after the inoculation of isolates of *Streptomyces* spp. colony diameter measurements were made, in three diametrically opposite directions, with the aid of a digital caliper, defining an average for each colony. The diameters of the pathogen colonies were compared with the control, whose pathogen developed in PDA medium. The mycelial growth inhibition percentage (GIP) was calculated using the formula by MENTEN *et al*. (1976), where: GIP = [(Control growth - treatment growth) / Control growth] x 100. The mycelial growth rate index (MGRI) was calculated according to the formula adapted by OLIVEIRA (1991): MGRI = Σ (D-Da) / N, where MGRI = Mycelial growth rate index ($cmday^{-1}$, D = Current average diameter (cm), Da = Average diameter of the previous day (cm), and N = Number of days after inoculation (days).

Statistical analysis: The data were subjected to analysis of variance F test and the Scott-Knott averaging cluster test at 5% probability using the statistical program ASSISTAT version 7.6 beta (SILVA, 2008).

RESULTS

Phenotypic characterization and phosphate solubilization: Based on the phenotypic characteristics observed, of the nine isolates only one (UFT-St08) presented slow growth, and all isolates acidified the culture medium, except for UFT-St01 (Table 1).

In relation to the colony, six isolates had a diameter greater than 2 mm, two isolates exhibited a flat colony and seven elevated colonies; all had a circular shaped colony and five had a transparent colony. In addition, all isolates presented positive results to produce filamentous and branched hyphae, and only one isolate (UFT-St03) did not present mycelium staining. As for the Gram stain test, it was observed that all isolates, except UFT-St01, presented Gram positive (Table 1). As for the evaluation of phosphate solubilization, which was carried out using a quantitative method, it was observed that only one isolate (UFT-St07) presented a solubilization index, being a medium index, that is, between 2 and 4 (Table 1).

In the presence of L-tryptophan an increase in the indole acetic acid synthesis is observed, ranging from 0.52 to 3.75 µg mL^{-1}. In 48 h of incubation, the best results are observed with the isolates UFT-St04 and UFT-St05, obtaining 8.5 and 8.7 µg mL^{-1}, respectively. However, these results are not maintained, since in the other incubation hours, better results were observed with the isolates UFT-St01 and UFT-St08, obtaining respective values of 2.89 and 3.28 µg mL^{-1} in 96 h; 3.62 and 3.16 µg mL^{-1} in 144 h; and 3.12 and 3.02 µg mL^{-1} in 192 h of incubation (Table 2). In a general average, it is observed that the isolates UFT-St08 and UFT-St09 were the best to produce IAA in the first hours of incubation, 0.95 and 1.71 µg mL^{-1} in 48 h, and 2.89 and 2.37 µg mL^{-1} in 96 h, respectively. With 144 and 192 h of incubation, the best isolates in the general average were UFT-St04 and UFT-St08, with 3.40 and 3.09 µg mL^{-1} in 144 h, and 3.80 and 2.98 µg mL^{-1} in 192 h, respectively. It is also important to note that the isolates presented more satisfactory results than those obtained with the control, both in the presence and in the absence of L-tryptophan (Table 2).

Table 1. Phenotypic characteristics of *Streptomyces* isolates

Isolates	Characteristics of isolates									
	TC	pH	DC	FC	EC	Tr	PH	CM	Gram	IS
UFT-St01	R	Al	≤ 2	C	E	N	S	P	-	-
UFT-St02	R	Ac	> 2	C	P	S	S	P	+	-
UFT-St03	R	Ac	> 2	C	P	S	S	A	+	-
UFT-St04	R	Ac	≤ 2	C	E	N	S	P	+	-
UFT-St05	R	Ac	> 2	C	E	N	S	P	+	-
UFT-St06	R	Ac	> 2	C	E	S	S	P	+	-
UFT-St07	R	Ac	> 2	C	E	S	S	P	+	++
UFT-St08	L	Ac	≤ 2	C	E	N	S	P	+	-
UFT-St09	R	Ac	> 2	C	E	S	S	P	+	-

TC- growth time (F: fast ≤ 3 days, S: slow> 3 days); pH of the medium (Ac: acid, Al: alkaline); DC - diameter of the colony in mm; SC - shape of the colony (C: circular); CE - colony elevation (F: flat, E: elevated); Tr - transparency (Y: yes, N: no); PH - production of filamentous and branched hyphae (Y: yes); MS - mycelium staining (P: present, A: absent); Gram - Gram test (+, Positive, -, Negative).> SI - solubilization index of P-Ca[+, SI <2 (low); ++, 2 SI 4 (medium); +++, SI 4 (high)].

Table 2. Production of IAA (µgmL^{-1}) by *Streptomyces* isolates in BD medium in the absence (AT) and presence (PT) of L-tryptophan.[1]

Isolates	Evaluation period (hours after prick)					
	48 hours			96 hours		
	AT	PT	Average	AT	PT	Average
Control	0.57 cA	0.52 dA	0.54 e	0.69 eA	0.65 fA	0.67 g
UFT-St01	0.66 cB	1.20 aA	0.93 b	1.04 dB	2.89 bA	1.96 c
UFT-St02	0.65 cA	0.57 dA	0.61 e	1.06 dA	0.72 fB	0.89 f
UFT-St03	0.54 cA	5.9 dA	0.57 e	0.73 eA	0.77 fA	0.75 g
UFT-St04	0.83 bA	8.5 cA	0.84 c	2.03 bA	1.83 dA	1.93 c
UFT-St05	0.78 bA	8.7 cA	0.82 c	1.37 cA	1.39 eA	1.38 d
UFT-St06	0.86 bA	5.9 dB	0.73 d	1.08 dA	1.31 eA	1.19 e
UFT-St07	0.55 cB	8.3 cA	0.69 d	0.67 eB	1.28 eA	0.97 f
UFT-St08	0.89 bA	1.00 bA	0.95 b	2.51 aB	3.28 aA	2.89 a
UFT-St09	2.20 aA	1.2 aB	1.71 a	2.58 aA	2.17 cB	2.37 b
Average	0.86 a	0.82 b	-	1.37 b	1.63 a	-
C.V.(%) [2]	9.6	9.6	9.6	9.9	9.9	9.9
	144 hours			192 hours		
Control	0.81 fA	0.73 fA	0.77 h	0.66 gA	5.92 fA	0.63 h
UFT-St01	1.03 eB	3.62 aA	2.32 d	0.98 fB	3.12 bA	2.05 d
UFT-St02	1.75 cA	0.78 fB	1.26 f	1.84 dA	0.74 fB	1.29 f
UFT-St03	0.73 fA	0.75 fA	0.74 h	0.57 gB	0.89 fA	0.73 h
UFT-St04	3.68 aA	3.12 bB	3.40 a	3.85 aA	3.75 aA	3.80 a
UFT-St05	1.36 dB	1.79 dA	1.57 e	1.30 eB	1.93 dA	1.62 e
UFT-St06	1.12 eA	0.90 fA	1.01 g	1.05 fA	0.83 fB	0.94 g
UFT-St07	0.69 fB	1.36 eA	1.03 g	0.69 gB	1.29 eA	0.99 g
UFT-St08	3.01 bA	3.16 bA	3.09 b	3.02 bA	2.94 bA	2.98 b
UFT-St09	2.91 bA	2.22 cB	2.56 c	2.60 cA	2.22 cB	2.41 c
Average	1.71 b	1.84 a	-	1.66 b	1.83 a	-
C.V.(%) [2]	7.9	7.9	7.9	7.6	7.6	7.6

[1] Averages followed by the same lowercase letter in the column and uppercase in the line, do not differ by Scott Knott's test at 5% significance. [2] Coefficient of variation.

Production of indole acetic acid: All isolates were able to synthesize IAA, even in small quantities, both in the presence and in the absence of L-tryptophan (Table 2). In the absence of the inducer, the IAA synthesis ranged from 0.54 to 3.85 µg mL^{-1}. In the first 48 and 96 h of incubation, better results with the isolates UFT-St08 (0.89 and 2.51 µg mL^{-1}) and UFT-St09 (2.20 and 2.58 µg mL^{-1}) were observed, in the evaluation of 144 and 196 h, the UFT-St08 isolate remained with the best results, increasing the production to 3.01 µgmL^{-1} in 144 h and maintaining the value in 196 h (3.02 µg mL^{-1}). The same did not happen with the UFT-St09 isolate. An increase in production is observed with the UFT-St04 isolate, being 3.68 and 3.85 µgmL^{-1} in 144 and 192 h, respectively.

Antagonistic activity: In relation to the pathogen *Curvularia lunata,* it was observed that the isolates of *Streptomyces* spp. presented inhibition, where the percentage of inhibition ranged from 29.9 to 91.7% (Table 3). The best percentages of inhibition were observed with the UFT-St05 and UFT-St07 isolates on all evaluation days, with

the best inhibition values being 90% for the UFT-St05 isolate (Figure 3E) and 91.7% for the isolate UFT-St07 with 96 h of incubation (Figure 3G). The lowest percentages of inhibition were observed with the UFT-St01 isolate (Figure 3A), reaching values less than 50% with 144 and 192 h of incubation, with 39.6 and 29.9% respectively. In addition, the mycelial growth evaluation points to reduced mycelial growth rate index (MGVI) values when compared to the control. A decrease in the index was observed according to the incubation time, with a maximum value of 9.2 with the isolate UFT-St01 in 48 h of incubation and values less than 1.2 for all isolates in 144 and 192 h of incubation. The data shows that the isolates that presented better results in relation to the growth inhibition percentage (GIP), also presented low values of MGVI, being 0.3 and 0.1 in 96 h of incubation for the isolates UFT-St05 and UFT-St07, respectively (Table 3).

In relation to the pathogen *Colletotrichum* sp., the percentage of inhibition ranged from 0.6 to 87.6% (Table 4). In 48 h of incubation, the best results of growth inhibition are observed with the isolates UFT-St07 and UFT-St08, with 60.8 and 58.6% inhibition, respectively. In the remaining hours of incubation, better inhibition is observed with the UFT-St04 and UFT-St07 isolates, with respective values of 72.1 and 76.6% in 96 h; 81 and 84.1% in 144 h; and 85.2 and 87.6% in 192 h. Thus, in general, the UFT-St07 isolate was the one that obtained the best results, exerting antagonistic action in relation to the pathogen, not allowing it to develop in the culture medium, which can be observed in Figure 4. The lowest percentages of inhibition are observed with the UFT-St03 and UFT-St06 isolates at all evaluated times (Table 4).

Table 3. Inhibition of mycelial growth of *Curvularia lunata* by isolates of *Streptomyces* spp.[1]

Treatments	Evaluation period (days after incubation)					
	48 hours			96 hours		
	Colony diameter (mm)	GIP[3] (%)	MGRI4	Colony diameter (mm)	GIP (%)	MGVI
Control	52.6 g		23.4	90.0 f		9.3
UFT-St01	24.1 f	54.2	9.2	48.7 e	45.9	6.1
UFT-St02	9.4 c	82.1	1.8	10.3 c	88.6	0.2
UFT-St03	15.9 e	69.8	5.1	20.4 d	77.3	1.1
UFT-St04	8.8 d	83.3	1.5	13.4 c	85.1	1.1
UFT-St05	7.9 b	85.0	1.1	9.0 b	90.0	0.3
UFT-St06	14.8 e	71.9	4.5	19.7 d	78.1	1.2
UFT-St07	5.6 a	89.4	0.0	6.2 a	93.1	0.1
UFT-St08	17.2 e	67.3	5.7	20.2 d	77.6	0.7
UFT-St09	16.9 e	67.9	5.6	22 d	75.6	1.3
C.V.(%)[2]	7.0			7.3		
	144 hours			192 hours		
	Colony diameter (mm)	GIP (%)	MGVI	Colony diameter (mm)	GIP (%)	MGVI
Control	90 e		0.0	90.0 e		0.0
UFT-St01	54.4 d	39.6	1.0	63.1 d	29.9	1.1
UFT-St02	14.6 b	83.8	0.7	20.0 b	77.8	0.7
UFT-St03	24.4 c	72.9	0.7	32.0 c	64.4	1.0
UFT-St04	15.4 b	82.9	0.3	19.2 b	78.7	0.5
UFT-St05	11.0 a	87.8	0.3	15.0 a	83.3	0.5
UFT-St06	23.7 c	73.7	0.7	28.6 c	68.2	0.6
UFT-St07	7.5 a	91.7	0.2	10.1 a	88.8	0.3
UFT-St08	26.5 c	70.6	1.1	33.6 c	62.7	0.9
UFT-St09	26.2 c	70.9	0.7	31.6 c	64.9	0.7
C.V.(%)[2]	6.8			11.4		

[1]Averages followed by the same letter in the column do not differ from each other by the Scott-Knott test ($p < 0.01$). [2]Coefficient of Variation. [3]GIP: Growth Inhibition Percentage. [4]MGVI: Mycelial growth velocity index.

Table 4. Inhibition of mycelial growth of *Colletotrichum* sp.by isolates of *Streptomyces* spp.[1]

Treatments	Evaluation period (days after incubation)					
	48 hours			96 hours		
	Colony diameter (mm)	GIP[3] (%)	MGRI4	Colony diameter (mm)	GIP (%)	MGVI
Control	18.6 c		5.8	31.2 e		3.2
UFT-St01	8.5 a	54.3	0.8	18.1 c	42.0	2.4
UFT-St02	8.8 a	52.7	0.9	8.8 a	71.8	0.0
UFT-St03	14.5 b	22.0	3.8	25.0 d	19.9	2.6
UFT-St04	8.7 a	53.2	0.8	8.7 a	72.1	0.0
UFT-St05	8.6 a	53.8	0.8	12.9 b	58.7	1.1
UFT-St06	17.9 c	3.8	5.4	31.0 e	0.6	3.3
UFT-St07	7.3 a	60.8	0.1	7.3 a	76.6	0.0
UFT-St08	7.7 a	58.6	0.4	15.1 b	51.6	1.8
UFT-St09	7.9 a	57.5	0.4	14.0 b	53.5	1.9
C.V.(%)[2]	8.2			8.7		
	144 hours			192 hours		
	Colony diameter (mm)	GIP (%)	MGVI	Colony diameter (mm)	GIP (%)	MGVI
Control	45.9 f		2.4	58.7 f		1.6
UFT-St01	19.6 c	57.3	0.2	19.6 c	66.6	0.0
UFT-St02	8.8 a	80.8	0.0	8.8 a	85.0	0.0
UFT-St03	31.4 d	31.6	1.1	34.4 d	41.4	0.3
UFT-St04	8.7 a	81.0	0.0	8.7 a	85.2	0.0
UFT-St05	12.9 b	71.9	0.0	12.9 b	78.0	0.0
UFT-St06	39.0 e	15.0	1.3	47.3 e	19.4	1.0
UFT-St07	7.3 a	84.1	0.0	7.3 a	87.6	0.0
UFT-St08	15.3 b	66.7	0.0	15.7 b	73.3	0.0
UFT-St09	14.2 b	69.1	0.0	15.1 b	74.3	0.0
C.V.(%)[2]	7.1			8.5		

[1]Averages followed by the same letter in the column do not differ from each other by the Scott-Knott test ($P < 0.01$). [2]Coefficient of Variation. [3]GIP: Growth Inhibition Percentage. [4]MGVI: Mycelial growth rate index.

As for the mycelial growth rate index (MGVI), similar results to those obtained in the study with *Curvularia lunata* are observed, since the MGRI values were low when compared to the control. In addition, according to the incubation time, there was a decrease in the growth rate, being less than or equal to 0.3 for all isolates at 144 h and 192 h of incubation, except for UFT-St03 and UFT-St06 (Table 4).

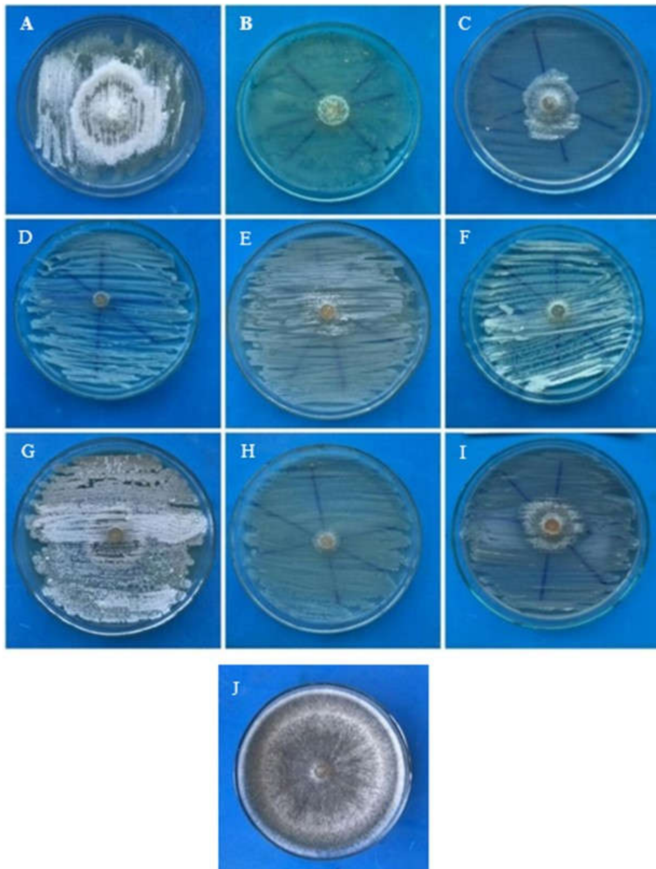

Figure 3. *In vitro* antagonistic activity with isolates of *Streptomyces* spp. Against the pathogen *Curvularia lunata*: A, UFT-St01; B, UFT-St02; C, UFT-St03; D, UFT-St04; E, UFT-St05; F, UFT-St06; G, UFT-St07; H, UFT-St08; I, UFT-St09; J, Control

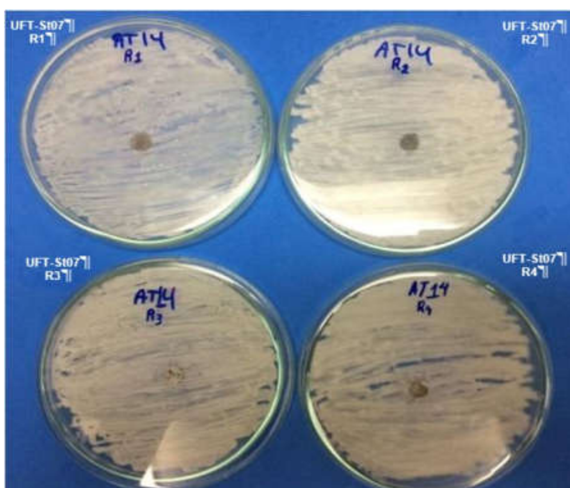

Figure 4. *In vitro* antagonistic activity with isolate of *Streptomyces* UFT-St07, againstthe *Colletotrichum* sp. Pathogen

DISCUSSION

According to the obtained results regarding the morphological characterization, the isolates studied were grouped into the genus *Streptomyces*, although one of them did not obtain a positive result regarding the gram stain test (Table 1). *Streptomyces* belong to actinomycetes, that is, gram positive bacteria. They are found primarily in the soil and their colonies exhibit varied colors, which can be identified by their opaque, rough, and non-disseminating morphology (MADIGAN et al., 2016). Oliveira (2003) describes that actinomycetes have varied morphological characteristics, making it possible to differentiate them from other bacteria with ease. Some of these characteristics are the production of mycelium, which causes them to present a powdery appearance, the production of filamentous and branched hyphae, and the production of pigments. It is important to note that the isolates presented quite varied phenotypic characteristics, which suggests great genetic diversity among them. In relation to the phosphate solubilization, the results showed that only one isolate presented a solubilization index (Table 1), but reports state that phosphate solubilizing bacteria can convert the insoluble form of phosphorus into a form available for plants. PANDE et al. (2017) eight bacterial isolates from the Nainital region in India were evaluated, obtaining three isolates with high phosphate solubilization. In addition, the gene sequence analysis of these three best isolates indicated that two of them were closely related to *Alcaligenes aquatilis* and one to *Burkholderia cepacia*.

Phosphate solubilization by soil bacteria is related to the lowering of the pH of the medium by organic acids, and the organic acids released by the bacteria can dissolve the mineral phosphate as a result of the exchange of anion from PO_4^{-2} to acid anion (HARA & OLIVEIRA, 2005). A study of solubilization by microorganisms, both fungi and bacteria, isolated from the rhizosphere of pigeon pea (*Cajanus cajan* L. Millsp.) Found that most fungi lowered the pH of the medium from 6.5 to values between 2.0 and 4.0, while for the bacteria, the variation was 4.0 and 6.5. This caused a higher average of solubilized phosphate to be observed with fungi (12 2mg L^{-1}) than with bacteria (15 mg L^{-1}). In addition, the authors state that the solubilized phosphate content depends on the type of species and the type of soil in which they were isolated (SOUCHIE & ABBOUD, 2007). As for the ability to synthesize IAA, all isolates in studies presented production, but being higher in the presence of L-tryptophan added to the culture medium (Table 2). According to Patten and Glick (1996), a microorganism has several routes for the synthesis of IAA and can choose one of them according to the environment. Different varieties of bacteria have the capacity to produce this auxin, by more than one route, most of them using tryptophan (GOSWAMI et al., 2015). Thus, this precursor has been added to the culture medium to promote increased synthesis (ZAKHAROVA et al., 1999). An example is the study by Tamreihao et al. (2016) evaluating the biocontrol and plant growth promotion activities of a strain of *Streptomyces corchorusii*, obtaining that the strain produced up to 30.5μgmL^{-1} of IAA and solubilized a significant amount of inorganic phosphate (up to 102 μgmL^{-1}). In addition, it produced 69% of siderophore units. In another study, it was observed that a strain of *Streptomyces* sp. acidotolerant isolated from limestone quarry produced 25 μg mL^{-1} of indole acetic acid, solubilizing up to 140 μg mL^{-1} of phosphate with a concomitant decrease in the pH of the medium (TAMREIHAO et al., 2016).

MU'MINAH et al. (2015), evaluated the production of exopolysaccharides and growth-stimulating substances, such as indole acetic acid in the presence of the precursor L-tryptophan by bacteria isolated from the rhizosphere of potato plants. The results showed 34 isolates producing IAA in the range of 0.40 to 21.14 mg L^{-1}. OLIVEIRA et al. (2012), in a study of the potential for production of indole acetic acid (IAA) by isolates of *Trichoderma* spp. Both in the presence and in the absence of L-tryptophan, they observed significantly greater results with the use of L-tryptophan, reaching values of 19.9 μg mL^{-1} on the sixth day of evaluation. But the authors noted that in the absence of the inducer, the isolates were also able to produce IAA. Similar results were observed in the study of KUSS et al. (2007) diazotrophic bacteria associated with rice roots without the use of the tryptophan inducer, in which there was IAA production by all isolates, with a range between 2.79 and 13.47 μg mL^{-1}. This fact is highlighted by Tsavkelova et al. (2006), who state that there are bacteria that can produce IAA in the absence of tryptophan, following the metabolic pathway that does not depend on this precursor.

However, the authors also state the mechanisms involved in the synthesis of IAA through this metabolic pathway are not yet fully elucidated. Thus, the factors identified as determinants to produce IAA are the species studied and the conditions of cultivation of the microorganism, such as the growth phase of the isolate, oxygenation, pH and the presence or absence of the inducer (PEDRAZA et al., 2004). As for the antagonistic capacity of *Streptomyces* isolates, the potential for the biocontrol of *Curvularialunata* and *Colletotrichum* sp., was evident (Tables 3 and 4). The genus *Streptomyces* is commonly mentioned as an excellent producer of several useful bioactive metabolites, such as antibiotics (NGUYEN et al., 2015). In addition to the production of antibiotics, *Streptomyces* also produce cell-wall degrading enzymes, such as chitinases and glucanase (KAUR et al., 2016). Thus, for these reasons, the genus *Streptomyces* has gained great importance as a biocontrol agent in the agricultural field, due to its potential to inhibit various fungal and bacterial pathogens.

An example of the potential of *Streptomyces* is portrayed in the study of Bressan and Figueiredo (2001), in which it was evaluated the effect of isolates of *Streptomyces* spp. in the *control of Stenocarpella maydis* under *in vitro* conditions and in corn seeds. The authors observed that under *in vitro* conditions, the isolates of *Streptomyces* spp. presented antagonistic effectiveness, and the zones of inhibition of the development of *Stenocarpella maydis* ranged from 30 to 52 mm. In relation to the seeds, those that were inoculated with *Streptomyces* had a reduction in the incidence of the fungus of about 90%, showing the effectiveness of the isolates in the control of *Stenocarpella maydis*. The biocontrol potential of *Streptomyces goshikiensis* against *Fusarium oxysporum* sp. Niveum was evaluated by Faheem et al. (2015), in which it was observed that the strain produced volatile antifungal compounds capable of inhibiting the growth of the fungus *in vitro* by up to 40%, in addition to a wide range of phytopathogenic fungi. The study by Singhand Gaur (2017) evaluated the induction of defense regulatory genes by *Streptomyces* spp. endophytic against *Sclerotium rolfsii* in chickpeas, in which it was observed that the strain regulates genes encoding enzymes such as superoxide dismutase, peroxidase, ascorbate peroxidase and guaiacol peroxidase, leading to an induced resistance against *S. rolfsii*.

CONCLUSIONS

The *Streptomyces* isolates presented varied phenotypic characteristics, only the UFT-St07 isolate presented a phosphate solubilization index. Regarding the production of indole acetic acid, the best results were obtained in the presence of the L-tryptophan inducer, and the best isolates observed were UFT-St04 and UFT-St08. The isolates UFT-St05 and UFT-St07 presented a positive effect on the biocontrol of *Curvularia lunata*., and the isolates UFT-St04 and UFT-St07 on the biocontrol of *Colletotrichum* sp.

REFERENCES

BADAWI, F. S. F.; BIOMY, A. M. M.; DESOKY, A. H. Peanut plant growth and yield as influenced by co-inoculation with *Bradyrhizobium* and some rhizo-microorganisms under sandy loam soil conditions. *Annals of Agricultural Sciences*, v. 56, n. 1, p. 17-25, 2011.

BRESSAN, W.; FIGUEIREDO, J. E. F. Potencial de isolados de *Streptomyces* spp. no controle de *Stenocarpella maydis* em sementes de milho. Embrapa - Comunicado Técnico, p. 1-4, 2001.

BREZA, B.; PALUSZAK, Z. The antagonistic activity of actinomycetes of *Streptomyces* genus in relation to *Trichoderma koningii. Journal of Ecological Engineering.* v. 17, n. 1, p. 106-113, 2016.

ESPAÑA, M. D.; ARBOLEDA, J. W.; RIBEIRO, J. A.; ABDELNUR, P. V.; GUZMAN, J. D. Eucalyptus leaf byproduct inhibits the anthracnose-causing fungus *Colletotrichum gloeosporioides*. Industrial Crops and Products, v. 108, p. 793-797, 2017.

FAHEEM, M.; RAZA, W.; ZHONG, W.; ZHANG, N.; QIRONG, S.; YANGCHUN X. Evaluation of the biocontrol potential of *Streptomyces goshikiensis* YCXU against *Fusarium oxysporum* f. sp. niveum. *Biological Control,* v. 81, p. 101-110, 2015.

GAO, S.; NI, X.; LI, Y.; FU, K.; YU, C.; GAO, J.; WANG, M.; LI, Y.; CHEN, J. Sod gene of *Curvularia lunata* is associated with the virulence in maize leaf. *Journal of Integrative Agriculture,* v. 16, n. 4, p. 874-883, 2017.

GORDON, S.A.; WEBER, R.P. Colorimetric estimation of indole acetic acid. Plant Physiology, Rockville, v. 26, n. 1, p. 192-195, 1951.

GOSWAMI, D.; THAKKER, J. N.; DHANDHUKIA, P. C. Simultaneous detection and quantification of indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) produced by rhizobacteria from l-tryptophan (Trp) using HPTLC. Journal of Microbiological Methods, v. 110, n. 2014, p. 7-14, 2015.

GOULART, A. C. P. (EMBRAPA A. O). Fungos em sementes de soja: detecção, importância e controle. *Embrapa Agropecuária* Oeste.p. 72, 2014.

GRAÇAS, J. P.; RIBEIRO, C.; COELHO, F. A. A.; CARVALHO, M. E. A.; CASTRO, P. R. C. E. Microrganismos Estimulantes Na Agricultura. Série Produtor Rural, v. 59, p. 56, 2015.

HARA, F. A. S.; OLIVEIRA, L. A. Características fisiológicas e ecológicas de isolados de rizóbios oriundos de solos ácidos e álicos de Presidente Figueiredo, Amazonas. *Acta Amazônica,* v. 34, n. 3, p. 343-357, 2004.

HARA, F. A. S.; OLIVEIRA, L. A. Características fisiológicas e ecológicas de isolados de rizóbios oriundos de solos ácidos de Iranduba, Amazonas. *Pesquisa Agropecuária Brasileira,* v. 40, n. 7, p. 667-672, 2005.

HARA, F. A. S.; OLIVEIRA, L. A. Características fisiológicas e ecológicas de isolados de rizóbios oriundos de solos ácidos de Iranduba, Amazonas. *Pesquisa Agropecuária Brasileira,* v. 40, n. 7, p. 667-672, 2005.

HERNANDEZ, R. A.; HEYDRICH-PEREZ, M.; ACEBO-GUERRERO, Y.; VELAZQUEZ, D. V.; M. G.; HERNANDEZ, L. N.A. Antagonistic activity of Cuban native rhizobacteria against *Fusarium verticillioides* (Sacc.) Nirenb. in maize (*Zea mays* L.). Applied Soil Ecology, Amsterdan, n. 39, v. 2, p. 180-186, 2008.

HUNGRIA, M.; CHUEIRE, L. M. O.; COCA, R. G.; MEGÍAS, M. Preliminary characterization of fast growing rhizobial strains isolated from soybean nodules in Brazil. Soil Biology and Biochemistry, v. 33, n. 10, p. 1349-1361, 2001.

KAUR, T.; JASROTIA, S.; OHRI, P.; MANHAS, R. K. Evaluation of in vitro and in vivo nematicidal potential of a multifunctional streptomycete, *Streptomyces hydrogenans* strain DH16 against *Meloidogyne incognita*. Microbiological research, v. 192, p. 247-252, 2016.

KONEMAN, E. W. WINN, JR. W.; ALLEN, S.; JANDA, W.; PROCOP, G.; SCHRECKENBERBER, P.; WOODS, G. Diagnóstico Microbiológico, Texto e Atlas Colorido. 6ª ed. Rio de Janeiro: Guanabara Koogan, 2008.

KUSS, A. V.; KUSS, V. V.; LOVATO, T.; FLÔRES, L. Fixação de nitrogênio e produção de ácido indolacético in vitro por bactérias diazotróficas endofíticas. PesquisaAgropecuaria Brasileira, Brasília, v.42, n.10, p.1459-1465, 2007.

LIMA, A. S. T. DE; BARRETO, M. DO C. S.; ARAÚJO, J. M.; SELDIN L.; BURITY, H. A.; FIGUEIREDO, M. V. B. Sinergismo *Bacillus*, *Brevibacillus* e, ou, *Paenibacillus* na simbiose *Bradyrhizobium*-caupi. Revista Brasileira de Ciencia do Solo, v. 35, n. 3, p. 713-721, 2011.

MADIGAN, M. T.; MARTINKO, J. M.; BENDER, K. S.; BUCKLEY, D. H.; STAHL, D. A. Microbiologia de Brock,14. ed. Porto Alegre: ArtMed, p.960, 2016.

MASSENSSINI, A. M.; TÓTOLA, M. R.; BORGES, A. C.; COSTA, M. D. Solubilização potencial de fosfatos mediada pela microbiota rizosférica de eucalipto cultivado em topossequência típica da zona da mata mineira. Revista Brasileira de Ciência do Solo, v. 39, n. 3, p. 692-700, 2015.

MENTEN, J.O.M.; MINUSSI, C.C.; CASTRO, C.; KIMATI, H. Efeito de alguns fungicidas no crescimento micelial de *Macrophomina faseolina* (Tass.) Goid. *in vitro*.Fitopatologia Brasileira, Brasília, v.1, n.2, p.57-66, 1976.

MU'MINAH; BAHARUDDIN; SUBAIR, H.; FAHRUDDIN. Isolation and Screening Bacterial Exopolysaccharide (EPS) from potato rhizosphere in highland and the potential as a producer Indole Acetic Acid (IAA). Italian Oral Surgery, v. 3, p. 74-81, 2015.

NGUYEN, L. T; SCHMIDT H. A.; VON, H. A.; MINH, B. Q. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution,* v.32, p. 268-274, 2015.

OLIVEIRA, A. G. DE.; JUNIOR, A. F. C.; SANTOS, G. R. DOS.; MILLER, L. O.; CHAGAS, L. F. B. Potential phosphate solubilization and AIA production of *Trichoderma* spp. *Revista Verde de Agroecologia e Desenvolvimento Sustentável,* v. 7, n. 3, p. 149-155, 2012.

OLIVEIRA, J. A. Efeito do tratamento fungicida em sementes no controle de tombamento de plântulas de pepino (*Cucumis sativus* L.) e pimentão *(Capsicum annum* L.). Dissertação (Mestrado em Fitossanidade) – Universidade Federal de Lavras, p. 111, 1991.

OLIVEIRA, M. F. Identificação e caracterização de actinomicetos isolados de processo de compostagem. Dissertação (Mestrado em Microbiologia Agrícola e do Ambiente) - Universidade Federal do Rio Grande do Sul, Porto Alegre, RS., p. 140, 2003.

PANDE, A.; PANDEY, P.; MEHRA, S.; SINGH, M.; KAUSHIK, S. Phenotypic and genotypic characterization of phosphate solubilizing bacteria and their efficiency on the growth of maize. *Journal of Genetic Engineering and Biotechnology,* v. 41, n. 2, p. 109-117, 2017.

PATTEN, C.; GLICK, B. R. Bacterial biosynthesis of indole-3-acetic acid. *Canadian Journal of Microbiology,* v. 42, n. 3, p. 207-220, 1996.

PEDRAZA, R. O. RAMÍREZ, M. A.; XIQUI, M. L.; BACA, B. E. Aromatic amino acid aminotransferase activity and indole-3-acetic production by associative nitrogenfixing bacteria. *FEMS Microbiology Letters,* v. 233, n. 1, p. 15-21, 2004.

RATÓN, T. M. O.; YANO, R.; GÁMEZ, O. R.; FLOH, E. I. S.; DÍAZ, M. J. S.; BARBOSA, H. R. Isolation and characterization of aerobic endospore forming *Bacilli* from sugarcane rhizosphere for the selection of strains with agriculture potentialities. World *Journal of Microbiology and Biotechnology*, v. 28, n. 4, p. 1593-603, 2011.

SHE, W.; SUN, Z.; YI, L.; ZHAO, S.; LIANG, Y. *Streptomycesalfalfae* sp. Nov. and comparisons with its closest taxa streptomyces silaceus, *Streptomyces flavofungini* and *Streptomyces intermedius*. *International Journal of Systematic and Evolutionary Microbiology,* v. 66, n. 1, p. 44-49, 2016.

SILVA, A. B.; LANDGRAF, P. R. C. Inoculação de bactérias produtoras de ácido 3-indol acético em plantas de alface (*Lactuca sativa* L.) inoculation of indole-3-acetic acid producing bacteria in lettuce plants., v. 11, n. 1, p. 89-96, 2017.

SILVA, F. de A.S. ASSISTAT. Versão 7.6 beta. Campina Grande, 2008.

SINGH, S. P.; GAUR, R. Endophytic *Streptomyces* spp. underscore induction of defense regulatory genes and confers resistance against *Sclerotium rolfsii* in chickpea. Biological Control, v. 104, p. 44-56, 2017.

SOUCHIE, E. L.; ABBOUD, A. C. D. S. Solubilização de fosfato por microrganismos rizosféricos de genótipos de Guandu cultivados em diferentes classes de solo. Semina: Ciências Agrárias, v. 28, n. 1, p. 11-18, 2007.

TAMREIHAO, K.; NIMAICHAND, S.; CHANU, S. B.; DEVI, K. A.; LYNDA, R.; JEENIITA, N.; NINGTHOUJAMA, D. S. Acidotolerant *Streptomyces* sp. MBRL 10 from limestone quarry site showing antagonism against fungal pathogens and growth promotion in rice plants. *Journal of King Saud University - Science,* v. 12, p. 250-268, 2016

TAMREIHAO, K.; NINGTHOUJAMA, D. S.; NIMAICHAND, S.; SINGH E. S.; REENA P.; SINGH S. H.; NONGTHOMBA U. Biocontrol and plant growth promoting activities of a *Streptomyces corchorusii* strain UCR3-16 and preparation of powder formulation for application as biofertilizer agents for rice plant. Microbiological Research, v. 192, p. 260–270, 2016.

TSAVKELOVA, E.A.; KLIMOVA, S.Y.; CHERDYNTSEVA, T.A.; NETRUSOV, A.I. Microbial producers of plant growth stimulators and their practical use: a review. *Applied Biochemistry and Microbiology,* v. 42, p. 117-126, 2006.

WILLIAMS, S.; SHARPE, M.; HOLT, J. Bergey's manual of systematic bacteriology. v. 4,1989.

ZAKHAROVA, E.A.; SHCHERBAKOV, A.A.; BRUDNIK, V.V.; SKRIPKO, N.G.; BULKHIN, N.S.; IGNATOV, V.V. Biosynthesisof indole-3-acetic acid in *Azospirillum brasilense:* insights from quantum chemistry. *European Journal of Biochemistry,* v. 259, p. 572-576, 1999.

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