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Characterization of actinobacteria from the semiarid region, and their antagonistic effect on strains of rhizobia

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The actinobacteria are the most abundant bacterial group in the soil, where they present different characteristics and antagonistic effects on other microorganisms. The objectives of this study were to characterize and evaluate the antagonistic effect of actinobacteria from the semiarid region on rhizobia from the same climatic region. Tests were performed to observe chromogenic and micro-morphological characteristics, tolerance to pH levels of melanin production, and use of carbon sources. This occurred due to the use of modified CDA culture media and basal medium supplemented with specific carbon sources, as well as the use of Ral color chat to observe the colonies. The *in vitro* antagonism on rhizobia was evaluated in yeast malt agar (YMA) culture medium for the observation of inhibition halo by actinobacteria. We observed different characteristics in relation to the color of aerial mycelium and reverse pigments; tolerance to media with acid and alkaline pH; and production of melanoid pigment occurred only in three strains. The morphological characteristics were described for genera *Streptomyces* and *Saccharothrix*, later confirmed by molecular sequencing. These genera were generalists in use of carbon sources, showing their physiological versatility. Regarding antagonism, nine strains of the genus *Streptomyces* were able to inhibit *in vitro* strains of *Rhizobium tropici* and *Bradyrhizobium yuanmingense* also coming from semiarid soils. The antagonism between actinobacteria and rhizobia can directly affect the symbiosis between nitrogen-fixing bacteria and leguminous plants. These results are pioneering in observing antagonism of these species of rhizobia under the climate condition of the semiarid region, and may contribute to agricultural biotechnology.

Key words: *Streptomyces*, soil, microbe interactions, Brazil, agricultural biotechnology, actinobacteria, antagonistic effect.

INTRODUCTION

Several mechanisms occur in the soil that influence the structure and composition of the soil communities, as well as intra and inter-specific interactions, for example, competition. The competitor microbial species often secrete compounds that affect the fitness of other species. This fact is characterized by antibiosis (Morris

and Blackwood, 2007). The actinobacteria are an example of organisms that antagonize microorganisms by means of secondary metabolites (Karlovsky, 2008; Tarkka and Hampp, 2008) and/or secretion of enzymes that inhibit the growth of other organisms in the soil (Suneetha and Zaved, 2011).

These filamentous microorganisms are gram-positive bacteria, and have a high content of guanine and cytosine in their DNA (Monciardini et al., 2002; Flärdh and Buttner, 2009). They are abundant and dispersed in different ecosystems; soil is their main habitat, where they perform important functions in soil fertility (El-Tarabily and Sivasithamparam, 2006; Jayasinghe and Parkinson, 2008). The genera *Streptomyces*, *Nocardia*, *Micromonospora*, *Actinoplanes* and *Streptosporangium* stand out in the edaphic environment (Anandan et al., 2016). The actinobacteria have crucial role in the decomposition of organic matter in ecosystems and thereby influence the cycling of nutrients in the soil (Mabrouk and Saleh, 2014), making them an excellent indicator of biological activity of the soil (Arifuzzaman et al., 2010). On the other hand, the literature contains records of the antagonistic effect of actinobacteria, *in vitro* and/or *in vivo* on other microorganisms (Parmar and Dufresne, 2011), for example, when they interact with rhizobia (Gregor et al., 2003; Mingma et al., 2014).

Rhizobia are bacteria responsible for biological nitrogen fixation (BNF) through the established symbiosis with leguminous plants (Zilli et al., 2010). These bacteria are of great importance for maintenance and restoration of ecosystems, being useful for the recovery of areas degraded by different impacts (Pontes et al., 2012). Soils with high population density of actinobacteria can inhibit the growth of rhizobia, negatively affecting plant nodulation and consequently reducing nitrogen fixation and plant productivity (Pereira et al., 1999; Parmar and Dufresne, 2011).

There are few studies that address the relationship of actinobacteria versus rhizobia, especially in isolates from semiarid regions. Thus, the aim of this study was to characterize the actinobacteria obtained in the Brazilian semiarid region and test their antagonistic effect *in vitro* on rhizobia strains from semiarid soils.

MATERIALS AND METHODS

Microorganisms

Fourteen strains of actinobacteria (Lima et al., 2014) obtained from samples of soil (AC 46, AC 49, AC 50 and AC 56) and leaf litter ((AC 3, AC 5, AC 10, AC 12, AC 13, AC 14, AC16, AC 38, AC 42 and AC43) at the Aiuaba Ecological Station (6°40' S & 40°10' W), located in the southwestern part of the municipality of Aiuaba, Ceará state, and five strains of rhizobia (L 4, L 7, L 9, L 16, L 18 and L 22) originating from the rhizosphere of leguminous plants (*Mimosa hostilis, Mimosa caesalpiniaefolia, Poincianella bracteosa* and *Erythrina verna*) (Pinheiro et al., 2014) in areas of Ceará (4°58' S and 39°1' W and 4°7' S and 38°14' W), and the state of Rio Grande do Norte (5°39' S to 35°58' W and 5°57' S to 36°39' W) were used in this study. All microorganism collection sites are located in the semiarid region of northeastern Brazil with prevailing vegetation of Caatinga, shallow soils rich in minerals, and an average annual rainfall of 400 to 800 mm. These microorganisms belong to the collection of the Environmental Microbiology Laboratory of Micro-organisms (LAMAB) of Federal University of Ceará, Department of Biology.

Morphological characterization

The actinobacteria strains were cultured in Casein Dextrose Agar (CDA) medium (0.5 g of K_2HPO_4, 0.2 g of $MgSO_4.7H_2O$, 0.01 g of $FeSO_4.7H_2O$, 2.0 g of dextrose, 0.2 g of casein, 15 g of agar, 1000 mL of distilled water, and pH 6.5 to 6.6) (Clark, 1965), for seven days at 28°C in a biochemical oxygen demand (BOD) chamber. The chromogenic characterization was performed by observing the aerial and reverse mycelium after growth of strains in Petri dishes, according to Wink (2012), with the use of a RAL color chart. The micro-morphological characterization was carried out by microcultivation according to Kern and Blevins (2003), with modifications. A blade and two pieces of cotton moistened with distilled water (sterile) were placed in sterilized Petri dishes. A cube of CDA culture medium measuring approximately 1 cm^3 (Clark, 1965) was placed on the blade. The strain was inoculated on the cube sides, covered with a sterile coverslip, while the dish was closed and incubated in a BOD chamber for 7 to 14 days at 28°C. After this time, the coverslip was removed and placed on a clean slide containing a drop of cotton blue stain. The slides were observed under a Zeiss Axioplan optical microscope (Leica DM750 M, Heerbrugg, Switzerland) with a 100x magnification to visualize the actinobacteria's characteristics (Miyadoh, 1997; Goodfellow et al., 2012).

Utilization of carbon sources

The use of carbon sources was analyzed according to Shirling and Gottlieb (1966), using mineral salts agar as basal medium. A solution was prepared by 10% of each carbon source (arabinose, sucrose, xylose, inositol, mannitol, fructose, rhamnose, raffinose and cellulose), with glucose (positive control) and water (negative control). After this, each dish with medium was sterilized and cooled to about 60°C, a concentration of 1% of each carbon source was added. These carbon sources were sterilized by a Millipore filter (0.45 µm). Finally, the strains were transferred to Petri dishes (triplicates) and incubated in a BOD chamber at 28°C for 10 days. The use of the sources was analyzed by the observation of increase (+) or no growth (-) in Petri dishes with medium.

Production of melanoid pigment

Strains were grown in tyrosine agar medium (Shinobu, 1958) with

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Table 1. ERIC and PCR primers used in this study.

S/N	Resistant gene	Oligos sequence	Amplicon size (bp)	Reference
1	ERIC1	5'-ATGTAAGCTCCCTGGGGATTCAC-3'	100- 5000	Tian-Xing (2011)
2	ERIC2	5'-AAGTAAGTGACTGGGGTGAGCG-3'		
3	P027F	5'-GAGAGTTTGATCCTGGCTCAG-3'	1400	Weisburg et al. (1991)
4	1492R	5'-ACGGTTACCTTGTTACGACTT -3'		
5	StrepB	5'-ACAAGCCCTGGAAACGGGGT-3'	519	Rintala et al. (2001)
6	StrepE	5'- CACCAGGAATTCCGATCT-3'		

and without tyrosine. The Petri dishes were incubated at 28°C in a biochemical oxygen demand (BOD) chamber for 10 days. The formation of melanoid pigment was considered (formation of dark-colored pigment) after growth in a Petri dishes with tyrosine, this is because the pigment formation in both media does not reflect the production of melanin.

Tolerance to different pH values

The test for growth of actinobacteria at different pH was carried out according to Kishore et al. (2012), modified by using the CDA culture medium (Clark, 1965). The medium was adjusted to pH 4.0, 5.0, 6.0, 7.0, 8.0 or 9.0. The strains were transferred to Petri dishes (triplicates) with a solid medium and incubated at 28°C for 10 days. The growth of the strains will indicate tolerance of the tested pH. The soil from which the actinobacteria had been collected was analyzed to determine the pH according to Donagema et al. (2011).

Genomic DNA extraction and analysis of genetic variability and amplification of 16S rRNA

Total genomic DNA was obtained from actinobacteria and rhizobia belonging to the microbiological collection with the Wizard® genomic DNA purification kit (Promega, Madison, WI, USA), following the manufacturer's instructions. The Wizard® genomic DNA kit is based on a four-step process.

The first step is the purification procedure, lysing of the cell and the nucleus; an RNase digestion step was included at this time. The cellular proteins are then removed by a salt-precipitation step, which precipitates the proteins but leave the high molecular weight genomic DNA in solution. Finally, the genomic DNA was concentrated and desalted by isopropanol precipitation. The oligonucleotides (Tabe 1), enterobacterial repetitive intergenic consensus (ERIC) were used to evaluate the genetic diversity of strains (Tian-Xing, 2011).

The polymerase reaction (PCR) was performed with a final volume of 25 µL, using 60 ng of DNA; 2.5 µL of a reaction buffer 10x; 0.75 µL of $MgCl_2$ (50 mM); 2 µL of dNTP (2.5 mM); 1 µL of forward oligonucleotide, and 1 µL of reverse oligonucleotide (both in concentration of 10 pmol); 0.25 µL of Taq polymerase enzyme (5 U/µL) and ultrapure q.s.p. water to 25 µL. Amplification was performed using the following steps: initial denaturation at 94°C for 2 min followed by 30 cycles at 94°C for 1 min, 50°C for 1.5 min and 68°C for 4 min, and final extension at 68°C for 10 min. The ERIC-PCR product was subjected to electrophoresis on 1.2% agarose gel in triton extraction buffer (TEB) buffer 0.5x stained with ethidium bromide and was visualized under ultraviolet light. For amplification of 16S rRNA, the primers 3 and 4 (Table 1) were for the rhizobia (Weisburg et al., 1991), and the oligos 5 and 6 (Table 1) for the actinobacteria (Rintala et al., 2001).

The final volume of the PC reaction was 25 µL and amplification conditions are presented in Table 2. The reaction was carried out in an AmpliTherm thermal cycler. The amplicons generated by the PC reactions were purified using ExoSAP (exonuclease I, shrimp alkaline phosphatase). Sequencing was performed by the Sanger method with the BigDye Kit ABI3100 and an Applied Biosystem sequencer. The genetic diversity of the isolates was assessed by ERIC-PCR markers. The sequences were compared with existing sequences in the GenBank database using the BLAST program available at the site National Center for Biotechnology Information (NCBI; www.ncbi.nlm.nih.gov).

Antagonistic test *in vitro* between actinobacteria and rhizobia

The antagonistic effect of actinobacteria on the rhizobia was evaluated according to the methods of Gregor et al. (2003). The strains of rhizobia were grown in glass tubes with liquid (without agar) YMA medium (10 g of mannitol, 0.5 g of K_2HPO_4, 0.2 g of $MgSO_4.7H_2O$, 0.1 g of NaCl, 0.5 g of yeast extract, 5 mL of bromothymol blue, 15 g of agar, 1000 mL of distilled water, and pH 6.8) (Vincent, 1970), and shaken (150 rpm) on a shaker orbital for about seven days. The actinobacteria were also grown in glass tubes, but with liquid CDA medium on a shaker orbital at the same speed and time period as foe rhizobia. The inhibition test was performed in Petri dishes (90 mm in diameter), divided into four quadrants with solid (with agar) YMA medium, plus 100 uL of YMA broth spread with rhizobia with a Drigalski handle. Then, 5 uL of CDA broth with actinobacteria was added. The Petri dishes (triplicate) were incubated for 5 days at 28°C in a BOD chamber. After this period, the plates were evaluated by observing the presence of inhibition zones, which were measured (cm) using a caliper rule.

Data analysis

The number of strains with and without growth at the different pH levels were subjected to the chi-square test with $p \leq 0.05$, based at pH 7. The radii of the inhibition halos were measured and then the inhibition area (πr^2) was calculated. These area values were log-transformed. The Shapiro-Wilk test was applied to test for normality, and then the data were subjected to analysis of variance (ANOVA) at $p \leq 0.05$ with the agricolae package software R®.

Table 2. PCR thermal cycle performed in this study.

Oligos	Step	Temperature (°C)	Time (min)	Number of cycle
ERIC1/ERIC2	Initial denaturation	94	2	30
	Denaturation	94	1	
	Annealing	50	1	
	Extension	68	4	
P027F/1492R	Initial denaturation	94	5	30
	Denaturation	94	0.40	
	Annealing	58	0.35	
	Extension	72	1.20	
StrepB/StrepE	Initial denaturation	98	5	30
	Denaturation	95	1	
	Annealing	54	1	
	Extension	72	2	

Table 3. Coloration of the colonies and spore morphologies of strains of actinobacteria from soil.

Strain	Color		Morphology
	Aerial Mycelium	Reverse Pigment	
AC 03*	Grey (RAL 9007)	Yellow (RAL 1002)	Spore chain hooked to looped
AC 05*	Brown (RAL 8024)	Brown (RAL 7030)	Spore chain straight to flexuous
AC 10**	White (RAL 9003)	Cream (RAL 9001)	Long chains of smooth spores
AC 12*	Green (RAL 7009)	Yellow (RAL 1012)	Spore chain hooked to looped
AC 13*	Brown (RAL 8007)	Brown (RAL 8012)	Spiral spore chain
AC 14*	Green (RAL 7002)	Yellow (RAL 7034)	Spiral spore chain
AC 16*	Green (RAL 7013)	Green (RAL 7013)	Spore chain straight to flexuous
AC 38*	Brown (RAL 8007)	Brown (RAL 4009)	Spore chain straight to flexuous
AC 42*	Brown (RAL 8008)	Brown (RAL 4009)	Spore chain to spiral
AC 43*	Cream (RAL 9001)	Cream (RAL 9001)	Spore chain hooked to looped
AC 46*	Beige (RAL 1015)	Beige (RAL 1015)	Smooth spore surface
AC 49*	Green (RAL 7006)	Green (RAL 7000)	Spore chain to spiral
AC 50*	Brown (RAL 8007)	Brown (RAL 8008)	Spore chain to spiral
AC 56*	Beige (RAL 1011)	Green (RAL 6013)	Spore chain straight to flexuous

*Genus *Streptomyces*; ** Genus *Saccharothrix.*

RESULTS

Chromogenic and morphological characteristics

The strains from the semiarid region had different characteristics (Table 3), in relation to color from aerial mycelium and reverse pigment. The predominant colors in the air mass were brown (5), green (4), beige (2), and only 1 each of grey, white and cream. The pigmentations of the reverse mycelium were brown (5), green (3), yellow (3), cream (2) and beige (1). After the microcultivation, it was observed that the actinobacteria had the following morphological characteristics: spore chain hooked to looped, spore chain straight to flexuous, long chains of smooth spores, spiral spore chain and smooth spore surface. These morphological traits are similar and

Table 4. Tolerance of the strains of actinobacteria to different pH ranges and melanoid pigment production.

Genera	Strain	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	Melanin pigment
Streptomyces	AC 03	+	-	-	+	+	-	+
Streptomyces	AC 05	+	+	+	+	+	+	-
Streptomyces	AC 12	+	+	+	+	+	+	-
Streptomyces	AC 13	+	+	+	+	+	+	-
Streptomyces	AC 14	+	+	+	+	+	+	-
Streptomyces	AC 16	+	+	+	+	+	+	-
Streptomyces	AC 38	+	+	+	+	+	+	-
Streptomyces	AC 42	+	+	+	+	+	+	-
Streptomyces	AC 43	-	-	-	+	+	-	-
Streptomyces	AC 46	+	+	+	+	+	+	-
Streptomyces	AC 49	-	-	+	+	+	-	+
Streptomyces	AC 50	+	+	+	+	+	+	-
Streptomyces	AC 56	+	+	+	+	+	+	-
Saccharothrix	AC 10	-	-	-	+	+	-	+

described for genera *Streptomyces* and *Saccharothrix*.

pH tolerance and melanin production

Table 4 shows the growth characteristics of the strains at different pH and the production of melanin. Regarding tolerance to extreme pH levels, the strains of the two genera had different behavior, except at pH 7.0 and 8.0, where all were able to grow in CDA medium with modified pH. The pH of the soil samples from which the actinobacteria were obtained had acid characteristics ranging from 4.5 to 6.3. Finally, melanoid pigment production occurred only in three strains (AC 3, AC 10 and AC 49) of actinobacteria.

Use of carbon sources

The strains of actinobacteria had wide use of carbon sources (Figure 1), with glucose being used by all strains tested. The strains of the genus *Saccharothrix,* used all the carbon sources except sucrose. However, the use of these sources by strains of the genus *Streptomyces* varied, with 85% for cellulose, 92% for sucrose and mannitol, and 100% for the other sources.

Molecular identification of microorganisms

The amplification profile ranged from 1 to 9 amplicons with sizes of 500 to 1400 bp for actinobacteria and 4 to 12 amplicons with sizes from 300 to 1400 bp for strains of rhizobia. After the molecular sequencing of actinobacteria and rhizobia, we observed the presence of actinobacteria of the genera: *Saccharothrix* and *Streptomyces* (Table 5). Regarding rhizobia, we identified two genera: *Bradyrhizobium* and *Rhizobium*.

In vitro inhibition of rhizobia

Only the actinobacteria strains of the genus *Streptomyces* had an antagonistic effect on *in vitro* rhizobial strains tested (Table 6). Of the 14 tested actinobacteria, nine presented antibiosis, and of five strains of rhizobia used, only two were inhibited. There was no statistical difference between the inhibition of actinobacteria ($F = 1.55$ and $P = 0.14$) and between the rhizobia strains inhibited ($F = 1.29$ and $P = 0.28$). However, *Rhizobium tropici* was inhibited by six strains of *Streptomyces*, particularly *Streptomyces graminisoli*, with 9.4 cm² inhibition halo. *Bradyrhizobium yuanmingense* was antagonized by seven strains of *Streptomyces*, particularly *Streptomyces* sp. with 6.8 cm² inhibition area.

DISCUSSION

Observation of the colors of the aerial mycelium mass and reverse side is one of the first methods used to distinguish isolates.

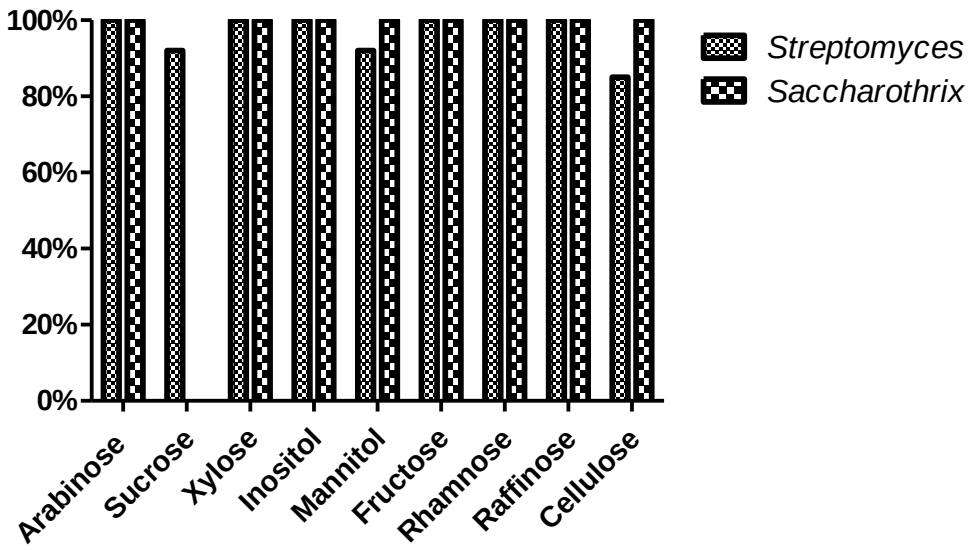

Figure 1. Percentage of use pattern of carbon sources for genera of actinobacteria identified.

Table 5. Molecular identification of strains of actinobacteria and rhizobia from semiarid soil by sequencing of 16S rDNA.

Bacteria	Strain	Number of Access GenBank	Species identity	Similarity %
Actinobacteria	AC 03	KY412816	*Streptomyces misionensis*	99%
	AC 05	KY412817	*Streptomyces lucensis*	99%
	AC 10	KY412821	*Saccharothrix* sp.	98%
	AC 12	KY412822	*Streptomyces graminisoli*	99%
	AC 13	KY412823	*Streptomyces sp.*	99%
	AC 14	KY412824	*Streptomyces misionensis*	98%
	AC 16	KY412825	*Streptomyces misionensis*	98%
	AC 38	KY412831	*Streptomyces griseoaurantiacus*	97%
	AC 42	KY412832	*Streptomyces corchorusii*	97%
	AC 43	KY412833	*Streptomyces sp*	97%
	AC 46	KY412835	*Streptomyces sp*	97%
	AC 49	KY412836	*Streptomyces* sp	94%
	AC 50	KY412837	*Streptomyces sp.*	97%
	AC 56	KY412839	*Streptomyces sp*	97%
Rhizobia	L 07	KY412842	*Bradyrhizobium elkanii*	98%
	L 09	KY412843	*Rhizobium tropici*	98%
	L 16	KY412844	*Bradyrhizobium japonicum*	97%
	L 18	KY412845	*Bradyrhizobium sp.*	97%
	L 22	KY412846	*Bradyrhizobium yuanmingense*	98%

In this study, brown was the predominant color, in contrast to those observed by Ramos et al. (2015) and Silva et al. (2015), who in characterizing actinobacteria strains coming from the semiarid region by cultures noted the predominance of gray, cream and white. This color variation is widely reported in the literature in actinobacteria strains originating from different regions, such as the Himalayas (Duraipandiyan et al., 2010), India (Kumar et al., 2010; Kumar et al., 2012; Das et al., 2014; Amsaveni et al., 2015), China (Yu et al., 2015), Egypt (Mabrouk e Saleh, 2014) and Iraq (Jaralla et al., 2014).

The color differences in the strains can be related to

Table 6. Inhibition zone area (cm) in vitro of antagonistic effect of actinobacteria on strains of rhizobia.

Strains	Actinobacteria	Inhibition area (cm^2) * of strains of rhizobia	
		Rhizobium tropici	***Bradyrhizobium yuanmingense***
AC 03	*Streptomyces misionensis*	6.4	NI
AC 05	*Streptomyces lucensis*	4.5	6.1
AC 12	*Streptomyces graminisoli*	9.4	NI
AC 13	*Streptomyces* sp.	5.1	6.8
AC 14	*Streptomyces misionensis*	6.5	5.2
AC 38	*Streptomyces griseoaurantiacus*	NI	2.2
AC 42	*Streptomyces corchorusii*	4.4	6.7
AC 43	*Streptomyces* sp.	NI	6.6
AC 50	*Streptomyces* sp.	NI	6.3
Coefficient of variation (CV) (%)		17.5	23.9

*Inhibition area calculated by πr^2; * NI, No inhibition.

factors such as temperature, soil type, pH and carbon sources in the environment (Amal et al., 2011). However, since the pigmentation of the colonies is similar among the different genera of actinobacteria, this feature would not be decisive for the strains classification.

Through microcultivation it was possible to distinguish two genera. This result of the spore chain through microcultivation corroborates the molecular identification analysis. This number was lower than that reported by Brito et al. (2015), who observed a similar genus (*Streptomyces*) to those found for this study. The genus *Streptomyces* was previously reported by Silva et al. (2013) in soils from the Brazilian Cerrado which is the most common in several regions (Anandan et al., 2016), with reports in the literature describing its occurrence in Atacama desert soils in Chile (Okoro et al., 2009), India (Kumar et al., 2012; Das et al., 2014) and Egypt (Mabrouk and Saleh, 2014).

Most actinobacteria strains did not produce melanoid pigments, but two (one of each genus), were able to produce melanin. Ramos et al. (2015) also observed this lower number of melanin-producing strains in strains coming from the semiarid region. Strains of *Streptomyces* derived from soil in India (Dastager et al., 2006) and Egypt (Mabrouk and Saleh, 2014) were characterized as not producing this pigment.

In mangrove sediments, Janaki et al. (2014) found a similar percentage of isolates capable of producing melanin as observed for us. This pigment is synthesized by fermentative oxidation, and has properties that can protect microorganisms against gamma and ultraviolet radiation (Amal et al., 2011; Manivasagan et al., 2013; Ahmed et al., 2014); this mechanism is against environmental stresses (Zhu et al., 2007; Manivasagan et al., 2013). However in our work, this mechanism was only used by some isolated actinobacteria from litter leaf (AC 3 and AC10), perhaps due to direct exposure to sunlight.

Regarding tolerance to pH, there was greater growth range of *Streptomyces* strains in media with pH 4.0, 7.0 and 8.0, but some strains were able to grow with other levels. However, the *Saccharothrix* strain only grew at pH 7.0 and 8.0. In soils of Colorado (USA), Lauber et al. (2009) found similar behavior to that of actinobacteria strains from the semiarid region of Brazil, an increase in pH from < 4.0 to > 8.0, while in coastal sediments in India, Ramesh and Mathivanan (2009) observed strains at pH 7.0 to 8.5, with most isolates between pH 8.1 to 8.5. But the variation in pH observed in the literature was different from that found in the soil from which actinobacteria were isolated. Actinobacteria are distributed in soils of different pH levels and are generally sensitive to extreme pH.

However, some genera can grow in acidophilus habitats (Anandan et al., 2016), with the most abundant microbial communities being found at pH < 4.1 (Rousk et al., 2010), and in alkaline soils (Meena et al., 2013). This allows actinobacteria to adapt and colonize different types of environments (Shivlata and Satyanarayana, 2015).

The carbon sources were used by both genera, except for sucrose by *Saccharothrix*. This general use has been reported in actinobacteria from marine sediments (Augustine et al., 2013; Meena et al., 2013) and soil of various habitats (Das et al., 2014; Jaralla et al., 2014.). Species within the genus are diversified regarding the use of sources, based on what the environment offers (Amal et al., 2011; Goodfellow et al., 2012.). This may explain the variation in some strains of the genus *Streptomyces*. Thus, the usage profile of sugars and alcohol (inositol) suggests potential generalist ability of the strains, and these differences in use may be due to the availability of carbon sources and adjustment of the isolates to various niches (Augustine et al., 2013).

The actinobacteria of the genus *Streptomyces* inhibited

in vitro rhizobia strains, forming inhibition zones ranging from 2.2 cm² to 9.4 cm², with antagonistic interaction with the species *Rhizobium tropici* and *Bradyrhizobium yuanmingense*. These two species of rhizobia, is known to nodulate some legume genera (*Phaseolus*, *Leucaena* and *Lespedeza*), vary in some traits, such as growth time (faster in *R. tropici* and slower in *B. yuanmingense*) and reaction in YMA culture medium (acid for *R. tropici* and alkaline for *B. yuanmingense*) (Martinez-Romero et al., 1991; Yau et al., 2002). The first record of antagonistic activity of actinobacteria against rhizobia was reported by Landerkin and Lochhead (1948), which test actinobacteria strains isolated from soil of the genus *Rhizobium*, and Van Schreven (1964) that compared actinobacteria of the genera *Streptomyces*, *Actinomyces* and *Nocardia* over *Rhizobium* strains. In *Bradyrhizobium japonicum*, Gregor et al. (2003) observed inhibition of these bacteria in *in vitro* growth by actinobacteria of the genera *Streptomyces* and *Amycolatopsis*. Mingma et al. (2014) isolated actinobacteria from roots and rhizosphere of leguminous plants which showed that, strains of the genus *Streptomyces* have negative effects on the growth of strains of *Rhizobium* sp. and *Rhizobium japonicum*.

There are reports in literature of such inhibition of rhizobia *B. japonicum* and *B. elkanii*, used as inoculants for soybean by actinobacteria of Brazilian Cerrado soils (Pereira et al., 1999). This work is the first reported observation of an antagonistic relationship, between actinobacteria and rhizobia coming from semiarid region soils from Brazil. The antagonistic inhibition occurs in the rhizosphere host with the release of secondary metabolites, enzymes and antibiotics (Karlovsky, 2008; Tarkka and Hampp, 2008; Suneetha and Zaved, 2011). The acquisition of nitrogen in some plants such as legumes often occurs by symbiosis with rhizobia, and the effect of inhibiting actinobacteria on rhizobia in the soil can be the cause of failure of nodulation process, and hence stunted plant growth (Tarkka and Hampp, 2008; Parmar and Dufresne, 2011).

Conclusion

Finally, results obtained expand knowledge of actinobacteria of the semiarid region of Brazil, and show the occurrence of an antagonistic effect exerted by these microorganisms on the rhizobia *R. tropici* and *B. yuanmingense*. This information can serve as a base for future studies, aiming to confirm this relationship and the *in vivo* effect which may contribute to agricultural biotechnology.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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