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Research Article

ISOLATION AND SCREENING FOR MULTI-TRAIT PLANT GROWTH PROMOTING ACTINOBACTERIA FROM ORGANIC SUGARCANE RHIZOSPHERE

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Abstract- Rhizospheric actinobacteria associated to organic cultivation are essential in nutrient cycling and plant growth promotion. The aim of this study was to isolate and select *in vitro* actinobacteria displaying multiple plant growth promoting traits associated with the rhizosphere of sugarcane undergoing organic management. The isolates were evaluated regarding their ability to produce plant growth promoting traits. Of the 21 isolates, 57% produced at least one of the evaluated traits. Isolates ABC92 and ABC32 produced 60.28 and 55.36 µg mL^{-1} of fitohormone in 21 days, whereas isolates ANC48 and ANU34 were the best solubilizers, solubilizing 8.93 and 8.92 µg mL^{-1} phosphate. A total of 29% of the microorganisms were able to grow in nitrogen-free media and 24% were ammonia producers. Isolates ABC31, ANC48 and ANU49 were able to inhibit *Fusarium moniliforme* growth. All actinobacteria identified in this study belonged to the *Streptomyces* genus and presented potential as plant growth promoting agents.

Keywords- *Rhizosphere, Organic Sugarcane, Phosphate Solubilization, Nitrogen Free Medium, Streptomyces*

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Introduction

In Brazil, although the conventional sugar cane production model is dominant, the acceptance and demand for organic agriculture products has driven the increase of areas destined for the organic production of this crop [1,2]. The organic production system does not use synthetic pesticides or fertilizers derived from petroleum, replacing them with animal and vegetable fertilizers, crop rotation and biological pest control. This type of system emphasizes the use of regional natural resources and the reuse of agricultural by-products, ensuring soil productivity, plant nutrition and pest control, minimizing negative environmental impacts, such as loss of biodiversity, nutrients, and soil degradation [3-6].

Organic agriculture leads to increased soil quality, as well as higher biodiversity, while it is dependent on the nutrient cycling conducted by microorganisms and their processes. As the contribution of organic matter to organic systems is high, microbial communities are essential in the transformation and release of nutrients necessary for plant growth [7-10]. In addition to their role in the nutrient cycling and as decomposers [11], soil microorganisms also display the potential for plant growth promotion [12-14].

Plant growth promoting rhizobacteria (PGPR), are rhizosphere-colonizing microorganisms, an intrinsic part of the soil adhered to the roots and widely influenced by root exudates, capable of contributing to plant health and productivity [15-18]. The mechanisms by which rhizobacteria can promote plant growth are classified as direct or indirect. Direct mechanisms involve the release of plant nutrients (phosphate solubilization and nitrogen fixation), release of various plant hormones (indole-3-acetic acid (IAA), gibberilines, cytokinins) and abiotic stress control (ACC-deaminase). Indirect mechanisms are related to plant protection against pests, through the production of substances with antimicrobial action or exoenzymes, the induction of systemic plant resistance and competition for nutrients and ecological niches [19-22].

Among the several microbial groups that inhabit the sugarcane rhizosphere, the most described correspond to gram-negative bacteria [23-26]. However, gram-positive microorganisms, especially those with high C+G content, such as actinobacteria, also present potential use as inoculants [27]. Members of the Actinobacteria phylum are commonly found in the rhizosphere, with a population of 10^6-10^9 cells per gram of soil [28] and are well known for their ability to produce enzymes and secondary metabolites [29, 30].

When in association with plants, actinobacteria have been described as important biocontrol agents, with the capacity to produce several antimicrobial substances [31-33]. The *Streptomyces* genus is the most described regarding actinobacteria capable of promoting plant growth, being associated with the production of IAA [34], phosphate solubilization [35], nitrogen fixation [36] and the production of enzymes and antifungal substances [37, 38].

Knowledge about the association between actinobacteria and organic systems, as well as their bioprospecting, is still poorly documented [39, 40]. In view of the close relationship between organic cultivation and nutrient release by soil microorganisms, actinobacteria are excellent prospecting targets.

Because they are mostly filamentous, they adhere to a greater extent to soil particles, maximizing nutrient release. In addition, they are sporulated and able to withstand adverse conditions, and are easily isolated and widely distributed in soils [11, 35]. In this context, this study aimed to carry out the isolation and selection, *in vitro*, of actinobacteria with multiple plant growth promotion characteristics associated with the rhizosphere of sugarcane under organic management from two sugarcane-processing lands located in the state of Goiás, Brazil.

Materials and Methods

Isolation of cultivable actinobacteria

Samples of rhizospheric soil were obtained from October 2015 to January 2016, originating from commercial sugarcane varieties IAC 911099 and CTC4 grown under an organic regime for sugar production. The sampled soils belonged to farms from two sugarcane-processing plants located in the state of Goiás, Brazil (19º 00' 023" S; 049º 40' 319" W and 15º 20' 241"S; 048º 54' 253" W). The plants were plucked from the soil and the aerial parts removed. The roots along with the adhered soil were put in sterile plastic bags and stored at 4 °C until processing, which occurred the day following the sampling.

The roots were vigorously shaken to remove excess soil. The rhizosphere soil was collected with the aid of a sterilized spatula, at a maximum distance of 5 mm from the roots [42]. The soil samples were heated at 40°C for 5 hours to facilitate actinobacteria isolation and to minimize the growth of other rapidly growing bacterial morphotypes.

One gram of rhizospheric soil was diluted in 100 mL of phosphate buffer (0.8% NaCl; 0.02% KCl; 0.14% Na_2HPO_4, 0.024% KH_2PO_4; pH 7.4) and maintained under stirring at 130 rpm for one hour. After incubation, the suspensions were serially diluted and concentrations of 10^{-3}, 10^{-4} e 10^{-5} were inoculated on Starch Casein Agar (SCA) [43] and agar ISP-2 [44] media, supplemented with 50 µg mL^{-1} of nystatin, to inhibit fungi growth [17].

The plates were incubated for 21 days at 30°C and monitored daily. The characteristic actinomycete colonies were transferred to a new culture medium and purified on the same isolation media. After purification, the actinobacteria were stored in 20% (w/v) glycerol and maintained at -20°C.

Screening for plant growth promotion abilities

IAA production

To evaluate the ability of the actinobacteria to produce IAA, the isolates were inoculated on ISP-2 agar and cultured for 14 days. Five mm plugs from the ISP-2 growth were inoculated into 100 mL tryptone yeast extract medium (YT) [45], supplemented with 5 mM L-tryptophan. The flasks were incubated under shaking at 130 rpm at 28°C for 21 days. After 7, 14 and 21 days, 2000 µL aliquots were centrifuged at 4,000 rpm for 30 minutes.

Determination of the IAA concentrations followed the colorimetric method described by Gordon and Weber [46]. The Salkowisk reagent (35% perchloric acid, 1.0 mL of 0.5M FeC_3) at a 1:1 (v: v) ratio was added to the supernatants. The solutions were then incubated for 30 minutes and absorbances were determined on a spectrophotometer at 530 nm. The results were expressed in µg mL^{-1} and determined from comparisons to a standard curve obtained from commercial IAA solutions (0 µg mL^{-1}; 1 µg mL^{-1}; 5 µg mL^{-1}; 10 µg mL^{-1}; 25 µg mL^{-1}; 50 µg mL^{-1}; 75 µg mL^{-1}; 100 µg mL^{-1}; 150 µg mL^{-1}; 200 µg mL^{-1}).

Phosphate solubilization

The ability to solubilize phosphate was evaluated by the method described by Nautiyal [47], with modifications. Five millimeters plugs from the previous actinobacteria growth for 14 days in ISP-2 medium were inoculated into flasks containing 100 mL of National Botanical Research Institute's phosphate growth medium (NBRIP) broth [47]. The samples were then incubated for 14 days at 28°C in a shaker under agitation at 130 rpm. After 7 and 14 days, 2000 µL aliquots were centrifuged at 10,000 x g for 5 minutes and the molybdate-vandate reagent (5.0% ammonium molibidate, 0.25% ammonium vanadate) was added to the supernatants as follows: 200 µL of supernatant, 200 µL of the reagent and 600 µL of distilled water [48]. Absorbances were determined on a spectrophotometer at 420 nm. The results were expressed in µg mL^{-1} and determined from comparison to a standard curve obtained from a KH_2PO_4 stock solution (0.0875%, m /v) and various soluble phosphate concentrations (0 µg mL^{-1}; 1.0 µg mL^{-1}; 5.0 µg mL^{-1}; 8.0 µg mL^{-1}; 10 µg mL^{-1}; 12 µg mL^{-1}; 15 µg mL^{-1}; 18 µg mL^{-1}; 20 µg mL^{-1}; 50 µg mL^{-1}; 75 µg mL^{-1}).

Growth in nitrogen-free medium

The isolates were initially cultured for 7 days in ISP-2 broth at 30°C in a shaker under agitation at 130 rpm. Growth aliquots were centrifuged at 10,000 xg for 10 minutes and the supernatants discarded. The cells were then resuspended in saline solution (0.85%, m/v), inoculated in the nitrogen-free semi-solid media, nitrogen-free bromothymol blue (NFb) [49] and Burk [50], and incubated at 30°C for 7 days. The microorganisms were harvested on new semi-solid media for 5 consecutive times to confirm growth capacity in a nitrogen-free environment.

Ammonia (NH_3) and hydrocyanic acid (HCN) production

The evaluation of NH_3 production followed the colorimetric method described by Cappuccino and Sherman [51]. The actinobacteria were inoculated in flasks containing 50 mL of peptone water and cultured for 7 days at 30°C under agitation at 130 rpm. After incubation, aliquots of the supernatant, were transferred to a microplate and Nessler's reagent (10% HgI_2; 7% KI; 50% aqueous NaOH 32%) was added to each well at a 2:1 (v/v) ratio. The positive control consisted of an ammonium sulphate solution (50 µg mL^{-1}). Ammonia production is evidenced by color changes, where the samples become brownish [52].

For HCN production, actinobacteria were inoculated on 10% tripticasein soybean (TSA) agar supplemented with 0.4% glycine. Filter papers (Whatman # 1) soaked with a picric acid solution (0.5% picric acid and 2.0% Na_2CO_3) were positioned under the plate covers. Plates were incubated for 14 days at 30°C. The development of a brownish color on the filter paper was indicative of HCN production [53].

Enzyme production: chitinases and cellulases

To evaluate chitinase production, actinobacteria were inoculated in nitrogen-free medium (NFM) supplemented with 8.0 g L^{-1} colloidal chitin, 0.78 g L^{-1} NH_4NO_3 and 15 g L^{-1} agar [54]. Plates were incubated at 30°C for 14 days. Chitinase producers were considered when a clear halo was observed around the colony. To verify cellulase production, actinobacteria were cultured in minimal medium [55] with the addition of 1.0% carboxymethylcellulose (CMC). After growth at 30°C for 14 days, the plates were treated with 0.3% Lugol solution [56]. After determining chitinase and cellulase production, the enzymatic index (EI) was calculated, corresponding to the ratio between the diameters of the halos by the diameters of the colonies.

Antagonistic activity against *Fusarium moniliforme*

The actinobacteria were evaluated concerning their ability to inhibit the growth of the phytopathogenic fungus *F. moniliforme*, according to the methodology adapted from El-Sayed, *et al*. [57]. They were inoculated into two parallel grooves and near the edges of a plate containing potato dextrose agar (PDA). After growth at 30°C for 7 days, 5 mm diameter disks from the previous growth of the fungus in PDA were deposited in the center of the plates. The plates were then again incubated at 30°C for 7 days and, when present, the inhibition halos were measured.

Statistical analyses

All tests were performed in triplicate and the results were submitted to analysis of variance (ANOVA). The means were compared by the Scott-Knott test at a 5.0% significance using the SISVAR® version 5.3 software [58].

Identification of multi-trait actinobacteria

Actinobacteria that during the *in vitro* screening were able to produce more than one plant growth promoting factor were identified by partial amplification of the 16S rRNA gene, after culturing in ISP-2 broth for 48 hours and shaking at 30°C and 130 rpm. DNA extraction was performed using isoamylic alcohol and chloroform protocol, according to the methodology described by Van Soolingen, *et al* [59].

The PCR reaction was prepared for 50 µL containing: 1 x Taq polymerase buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 mM of each primer, 2.5 U of Taq DNA polymerase and 1.0 µL of template DNA (50 ng). The oligonucleotide primers 27F (5'-AGAGTTTGATCLanCTGGCTC AG-3') and 1541R (5'-AAGGAGGTGATCCAGCC-3') were used for amplification [60].

The amplification of the genetic material was carried out in a thermal cycler, by initial denaturation at 94 ºC for 2 minutes, 30 denaturation cycles at 95 °C for 1 minute, annealing at 55 °C for 1 minute and an extension step at 72 ° C for two minutes. A final extension was performed at 72 °C for 10 minutes. The generated amplicons were evaluated regarding integrity on 1.2% agarose gels and purified with isopropanol and ethanol method [61]. Sequencing was conducted on the ABI 3130xl platform (Applied Biosystems), using the following oligonucleotide primers: 27F, 530F (5'-GTGCCAGCMGCCGCGG-3'), 519R (5'-GWATTACCGCGGCKGCTG-3'), 907R (5'-CCGTCAATTCMTTTRAGTTT-3'), 926F (5'-AAACTYAAAKGAATTGACGG-3') and 1541R [60].

The obtained sequences were checked for quality and joined using the CodonCode Aligner® version 6.0.2 software. Sequence identification was carried out by comparison to the available sequences at the NCBI (National Center for Biotechnology Information) database via the BLAST algorithm [62]. After identification, the sequences were deposited at the NCBI 16S rRNA database under the following accession numbers: MG388303, MG494375, MG494376, MG388199, MG388202, MG494383, MG388200, MG388201, MG494377.

For phylogeny purposes, the sequences were aligned with the ClustalW tool [63] of the MEGA software 7.0.212 [64] and dendrograms were constructed using the neighbor-joining method [65], according to the Jukes-Cantor model [66] with 1000 bootstrap replicas.

Results and Discussion

Twenty-two actinobacteria were isolated from the rhizosphere of sugarcane undergoing organic management, 58% from the CTC4 variety and 42% from the IAC1099 variety. The production of at least one of the *in vitro* plant growth promotion traits was observed in 57% of the isolates [Tables 1 and 2]. The occurrence of actinomycetes in the rhizosphere is quite common, being constantly associated with the ability to promote plant growth, either by increasing nutrient availability or by acting in biocontrol [67, 68]. The literature is divergent as to the number of cultivable actinomycetes recovered from organic systems. A study conducted by Wang, *et al*. [69] found that the amount of phospholipid-derived fatty acids (PLFA) from actinobacteria was higher in systems with higher organic matter input. On the other hand, Velmourougane [40] did not observe any differences in the number of cultivable actinobacteria between organic and conventional systems.

Table 1- *In vitro* production of direct plant growth promotion factors by actinobacteria isolated from sugarcane under organic management[1].

Isolates	IAA (µg.mL^{-1})			Phosphate solubilization (µg.mL^{-1})		Growth in nitrogen-free medium[3]
	7 days	14 days	21 days	7 days	14 days	
ABC21*	-	3.94^{c}	13.73^{c}	2.40^{b}	3.58^{b}	B
ABC31*	2.52^{b}	27.20^{b}	29.00^{b}	1.60^{b}	3.08^{b}	B
ABC32*	25.95^{a}	50.45^{a}	55.36^{a}	1.92^{b}	2.33^{c}	B
ABC33*	-	3.13^{c}	5.51^{d}	1.23^{b}	2.04^{c}	B
ABC92*	35.99^{a}	42.43^{a}	60.28^{a}	5.20^{a}	3.97^{b}	B
ABU33#	6.35^{b}	23.21^{b}	32.24^{b}	-	-	B
ANC21*	-	-	-	-	-	-
ANC48*	-	4.64^{c}	32.18^{b}	5.12^{a}	8.93^{a}	N/B
ANU48#	-	-	-	-	-	-
ANU34#	-	-	5.33^{d}	6.39^{a}	8.92^{a}	N
ANU49#	3.12^{b}	3.80^{c}	17.95^{c}	1.55^{b}	3.90^{b}	N
ANU50#	-	-	-	-	-	-

1 Means followed by the same letters do not differ statistically by the Scott-Knott test at a 5% probability. 2. (*) Actinobacteria isolated from the CTC4 variety; (#) actinobacteria isolates of the IAC 911099 variety. 3. Growth in nitrogen-free medium: B = Burk and N = NFB.

IAA production by actinomycetes ranged from 2.52 to 35.99 µg mL^{-1} after 7 days, 3.13 to 50.45 µg mL^{-1} after 14 days and 5.33 to 60.28 µg mL^{-1} after 21 days [Table-1]. The highest IAA yields were observed at 21 days, by isolates ABC92 and ABC32. For isolates ABC21, ABC33 and ANC48, IAA production was only detected at 14 days, while the production of isolate ANU34 was detected at 21 days, indicating late production of this hormone. The results obtained in this study were like those reported by Anwar, *et al* [17] when comparing the same L-tryptophan concentrations and the same incubation time.

In general, IAA production by actinobacteria is moderate when compared to other plant-associated bacterial phyla [70, 71]. However, the constant release of IAA, even in low soil concentrations, is responsible for lateral growth and development of adventitious roots. In addition to its role in plant tissue growth, IAA can also act as a signal for the production of secondary metabolites and actinobacteria sporulation [72, 73, 74].

Table 2- *In vitro* production of indirect plant growth promotion factors by actinobacteria isolated from sugarcane under organic management[1].

Isolate	HCN	NH_3	Chitinase	Cellulase	Antagonism
ABC21*	-	-	-	1.33^{c}	-
ABC31*	-	-	-	2.36^{b}	5^{a}
ABC32*	-	-	-	3.71^{a}	-
ABC33*	-	+	+	1.58^{c}	-
ABC92*	-	+	+	2.79^{b}	-
ABU33#	-	-	-	1.24^{c}	ami
ANC21*	-	-	-	1.38^{c}	-
ANC48*	-	+	+	2.66^{b}	4^{a}
ANU48#	-	-	-	2.84^{b}	-
ANU34#	-	+	+	2.02^{b}	ami
ANU49#	-	+	+	2.43^{b}	4^{a}
ANU50#	-	-	-	2.00^{b}	-

1. Means followed by the same letters do not differ statistically by the Scott-Knott test at a 5% probability. 2. (*) Actinobacteria isolated from the CTC4 variety; (#) actinobacteria isolates of the IAC 911099 variety. 3. The expressed values correspond to the enzymatic index obtained by dividing the size of the halo by the colony. The (-) label indicates bacterial growth without halo formation. 4. The presented values correspond to the distance between the fungal and bacterial colony. The marking (-) indicates that the bacterium was not antagonistic to the fungus. Ami = aerial mycelium inhibition.

Phosphate solubilization was evaluated during two periods, ranging from 1.23 to 6.39 µg mL^{-1} after 7 days and 2.04 to 8.93 µg mL^{-1} after 14 days [Table-1]. The best results were observed for the ANC48 and ANU34 isolates. It should be noted that the best phosphate solubilizers were not the best IAA producers. Jog, *et al*. [34] also found rhizosphere actinobacteria capable of solubilizing phosphate and concomitantly producing IAA. These authors reinforce the idea that bacteria participating in nutrient cycling are interesting in the choice of an inoculant candidate.

Even though phosphate solubilization is influenced by plant exudates [75], no statistical differences were observed in the present study between the phosphate solubilization capability of isolates from different varieties [Table-1]. The findings regarding the amount of phosphate solubilized by actinobacteria in the literature are quite variable [34, 35, 67, 76], and may be related to the type of technique, the source of the phosphorus or the units in which the phosphate solubilization is expressed [77]. Thus, some *in vitro* values obtained in this and other studies may have been underestimated.

Phosphorus is an essential element for plant growth and is rapidly converted into insoluble forms in soil. Phosphate solubilizing bacteria can react with phosphate binders, such as aluminum, iron, calcium and magnesium, releasing phosphate for plant use [78-80]. Among the various mechanisms responsible for phosphate solubilization in bacteria, the most common among actinobacteria is the production of organic acids, such as pyruvate, succinate, malate, lactate, α-ketoglutarate and oxalate [81, 82]. Growth capacity in nitrogen-free media was observed in 29% of the isolates, and most isolates grew in Burk's medium [Table-1]. NFb and Burk's media were not developed for actinobacteria isolation [49, 50], although they can be used in the screening of the diazotrophic potential of bacteria of this phylum. In this sense, the semi-solid media favors the screening of diazotrophic microorganisms since nitrogen fixation by most organisms requires low concentrations of molecular oxygen [83].

Nitrogen is one of the most important elements in the formation of biological macromolecules. Therefore, its lack limits several crops, especially organic crops, where the use of synthetic nitrogen compounds is not recommended [84]. In this regard, it is essential to prospect for nitrogen-fixing microorganisms. These organisms, also called diazotrophs, are able to convert nitrogen gas into ammonia, which is then absorbed by plants [85, 86]. Among actinobacteria, the *Frankia* genus is well known for its ability to fix nitrogen, both in free life and in symbiosis [87]. In addition to the *Frankia* genus, several other actinobacteria genera have been reported as being able to grow in nitrogen-free media, such as *Mycobacterium* [88], *Micromonospora* [89], *Agromyces* [88] and *Streptomyces* [90]. The use of semi-solid nitrogen-free media is the basis for the isolation of diazotrophic bacteria. However, it is necessary to confirm and quantify the amount of nitrogen that is fixed. Acetylene reduction, N^{15} incorporation and *nif*H gene amplification techniques have been applied to confirm and quantify nitrogen fixation [91, 92]. Regarding the *Streptomyces* genus, only one recent report is available, by Dahal, *et al* [36], confirming the ability of the genus to fix nitrogen through *nif*H gene amplification and N^{15} incorporation.

Although actinomycetes are IAA producers, solubilize phosphate and fix nitrogen, the literature describes them mainly as bioactive compound producers, producing antimicrobials and enzymes [93, 94]. In relation to indirect plant promotion mechanisms, none of the isolates were able to produce HCN and chitinase [Table-2]. Ammonia production was detected in 24% of the isolates [Table-2]. Damle and Kulkarni, [95] when isolating and screening actinobacteria capacity from the medicinal plant *Withania somnifera*, detected several *Streptomyces* strains capable of producing NH_3. Ammonia would have two roles in promoting plant growth, assisting in plant biomass increases [96] and acting in the defense against opportunistic pathogens [97]. Cellulase production was observed in 57% of the evaluated actinobacteria. The observed EI ranged from 1.24 to 3.71, with the highest index observed for isolate ABC32 [Table-2]. It is worth mentioning that, from all characteristics evaluated in this study, cellulase production presented the largest number of isolate producers. The production of hydrolytic enzymes by actinomycetes is quite common, and the *Streptomyces* genus is the most commonly reported in this regard [98-100]. The production of these enzymes would be primarily linked to actinobacteria roles as decomposers. These enzymes also participate in protection against pathogens, since they aid the cell wall breakdown process of fungal and bacterial pathogens, whose composition includes compounds such as chitin, glucan and cellulose [82, 101].

Table 3- Molecular identification of actinobacteria isolated from the rhizosphere of sugarcane under organic management.

Isolate	Identification	Similarity	Accession number of the nearest species	Deposit number
ABC21	*Streptomyces griseoplanus*	99%	KT820005.1	MG388303
ABC31	*Streptomyces* sp.	98%	NR_043346.1	MG494375
ABC32	*Streptomyces* sp.	92%	KX010107.1	MG494376
ABC33	*Streptomyces* sp.	99%	DQ663186.1	MG388199
ABC92	*Streptomyces* sp.	99%	KJ995861.1	MG388202
ABU33	*Streptomyces* sp.	99%	AB448717.1	MG494383
ANC48	*Streptomyces* sp.	98%	AB695175.1	MG388200
ANU34	*Streptomyces* sp.	98%	HQ876061.1	MG388201
ANU49	*Streptomyces* sp.	98%	KX618421.1	MG494377

1. Deposit number at the NCBI 16S rRNA gene database.

The ability to inhibit the growth of the pathogenic fungus *F. moniliforme* was observed for 24% of the isolates. Isolates ABC31, ANC48 and ANU49 were able to completely inhibit fungal growth, whereas ABU33 and ANU34 isolates only inhibited aerial mycelium formation [Table-2]. Kruasuwan and Tamchaipenet, [76] when studying bacteria isolated from sugarcane roots, also detected actinobacteria capable of inhibiting the *F. moniliforme* growth. The results of the present study are very promising, given that the use of synthetic antifungals is not allowed in organic cultivation, so the application of these microorganisms is a possible form of biological control.

The *Streptomyces* genus has been the most described regarding the production of antifungal metabolites against several pathogenic *Fusarium* sp. strains, reported in banana [102], cucumber [103] and ornamental plants [104]. The probable mechanism of action of this genus against pathogenic fungus is related to the production of extracellular metabolites that cause hyphal tumors and distortion, leading to the lysis of these structures. In addition, these metabolites promote spore germination inhibition [102].

Isolates capable of *in vitro* producing more than one plant growth promoting factor were identified by the partial sequencing of the 16S rRNA gene. All actinobacteria were identified as belonging to the *Streptomyces* genus [Table-3]. The phylogenetic relationships of the isolates indicate that all belonged to this genus, albeit, representing distinct species [Fig.-1], according to the different morphotypes observed during their isolation. It was possible to identify isolate ABC21 at the species level, identified as *Streptomyces griseoplanus*, with 99% similarity [Table-3]. The identification is reinforced by the phylogenetic affiliation of the isolate, supported by a 96 bootstrap value [Fig-1].

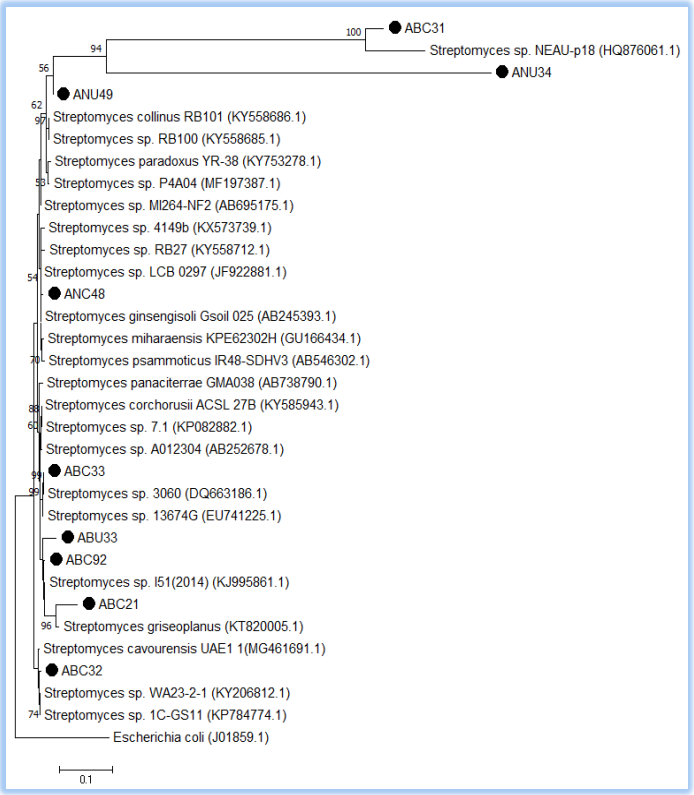

Fig.1- Phylogenetic tree based on the 16 rRNA gene sequences of actinobacteria from the rhizosphere of sugarcane under organic management. The Neighbor-Joining method was used with 1000 replicates. The bar indicates 0.01 substitutions per nucleotide position. Only bootstrap values above 50% are shown.

Actinobacteria phylum have been described as one of the most frequently associated with sugarcane-cultivated soils [105] and their rhizospheres [106]. The *Streptomyces* genus is the most numerous and studied within the Actinobacteria phylum, and has been employed in several plant growth potential studies [107, 108, 109].

Conclusion

The nine isolates identified in this study present not only potential as biocontrol agents but are also interesting from the point of view of plant nutrient release, since most of them are able to grow in nitrogen-free media, solubilize phosphate and produce IAA. Although the dynamics between microorganisms and plants in organic systems is poorly understood, prospecting studies such as the one presented here are extremely necessary, considering that one of the pillars of this production system is the optimum use of natural resources. Future studies on the application of these organisms in greenhouses and in the field are essential to quantitatively verify improvements in plant production. In addition, it is suggested that these microorganisms be used in association in organic systems.

Application of research: The present work could be applied to the production of desirable bacterial inoculants for crop production.

Abbreviations: analysis of variance (ANOVA), carboxymethylcellulose (CMC), Enzymatic Index (EI), indole-3-acetic acid (IAA), hydrocyanic acid, National Botanical Research Institute's phosphate growth medium (NBRIP), National Center for Biotechnology Information (NCBI), nitrogen-free bromothymol blue (NFb), nitrogen-free medium (NFM), phospholipid-derived fatty acids (PLFA), plant growth promoting rhizobacteria (PGPR), potato dextrose agar (PDA), Starch Casein Agar (SCA), trypticase soy agar (TSA).

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Author Contributions: All author equally contributed.

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