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MICROBIOLOGY

Autochthonous endophytic bacteria from *Musa* sp. controls *Fusarium oxysporum* f. sp. *cubense* under *in vitro* conditions

MARIA J. MARTINS, ADELICA A. XAVIER, ISABELLE C. CARDOSO, DANIELA F. SILVEIRA, REGINA C.F. RIBEIRO, SAMY PIMENTA & SILVIA NIETSCHE

Abstract: Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* (*foc*) is one of the main diseases affecting banana crops. Biological control emerges as an alternative technology to prevent the spread of the disease. The objective of this work was to evaluate the effects of endophytic bacteria isolated from banana Prata Anã challenged with the *foc* in pairing and volatile tests under *in vitro* conditions. Forty endophytic isolates of the genera *Bacillus*, *Klebsiella*, *Paenibacillus*, *Stenotrophomonas*, *Lysinibacillus* and *Sporolactobacillus* isolated from banana roots were challenged with *foc*. The principal component analysis showed that the spore germination variable in the presence of bacterial cells explained better the variance (29.88%). Spore germination in the presence of bacterial cells, number of spores/cm^2 in paired and volatile tests, and colony area in volatile tests explained about 86.10% of the total variance observed. The isolate EB37 (*Bacillus* sp., JN215502.1) reduced 96% of the germination of *Fusarium oxysporum* f. sp. *cubense* spores. The UPMGA clustering method based on Euclidean distance divides the 40 endophytic bacteria isolates into eight groups. The autochthonous bacteria isolated from *Musa* sp. of the genera *Bacillus*, *Lysinibacillus*, *Stenotrophomonas*, *Sporolactobacillus* and *Paenibacillus* showed promising results in *foc* control under *in vitro* conditions.

Key words: Fusarium wilt, biological control, *Bacillus* spp., spore germination, PCA.

INTRODUCTION

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cubense*, is one of the main diseases that occurs in the world's banana production and causes drastic economic losses to farmers (Fu et al. 2017). The fungus penetrates banana roots and invades the vascular tissue, causing symptoms of gradual wilt, progressive yellowing, and longitudinal division of leaf sheaths in the pseudo stem and, in more advanced stages, plant death (Yin et al. 2011).

Integrated control measures, with an emphasis in preventive measures including cultivating resistant cultivars and cultural controls have enabled living with the disease in the field and minimizes losses. The use of biological agents to control Fusarium wilt is a practice that has intensified in recent years, among them endophytes and strains of *Trichoderma* spp., *Pseudomonas* spp., and *Bacillus* spp. have been the most studied. In Brazil, the fungus *Trichoderma* spp. has been registered by the Ministério da Agricultura, Pecuária e Abastecimento (MAPA) to control phytopathogens of the genus *Fusarium* spp., and there is no official record reporting the use of endophytic bacteria isolated from banana cultivars to control the spread of Fusarium wilt.

However, several research studies have shown that the banana microbiome is quite diverse and rich in bacterial species (Souza et al. 2013, Pereira et al. 2018).

The microbiome study of healthy and diseased banana plants has demonstrated greater diversity in the healthy plant microbiome compared to the diversity observed in diseased plants, suggesting action in the suppression of root pathogens (Korbel et al. 2017). A recent study also demonstrated that components like rhizosfere, roots, and corms impacted bacterial communities in banana with more diversity in rhizosfere. Several of the communities discovered in banana presents potential for plant growth and health (Kaushal et al. 2020). Thus, it is assumed that the selection of autochthonous microorganisms is more effective for use in environments with the same bioecological characteristics as its selection. In banana Prata Anã cultivar, several species of *Bacillus* spp. were isolated, and the biotechnological potential was assessed and confirmed (Andrade et al. 2014, Matos et al. 2017, Gomes et al. 2017, Souza et al. 2017).

Endophytic microorganisms can produce toxins, antibiotics and secondary metabolites such as iturins, that act by inhibiting the germination of phytopathogens (Chen et al. 2018) and surfactin, that forms biofilms and prevents the host's recognition by the phytopathogen (Carrer Filho et al. 2015). Endophytic bacteria are also effective producers of volatile, liposoluble compounds and therefore have high solubility in fungal membranes (Fadiji & Babalola 2020).

The biocontrol of *F. oxysporum* f. sp. *cubense* and nematodes by bacteria associated with the banana microbiome has been described elsewhere (Ribeiro et al. 2012, Bidabadi & Sijun 2018). Bioprospecting isolates that could be candidates for biological control with multiple biological functions can increase biocontrol since they may indirectly act in promoting growth.

The present study aimed to evaluate 40 endophytic isolates from banana Prata Anã cultivar and select the most promising for biocontrol action on *Fusarium oxysporum* f. sp. *cubense*.

MATERIALS AND METHODS

Genetic materials, experimental site and preliminary procedures

Assays were carried out with *Fusarium oxysporum* f. sp. *cubense* isolate 106 (race 1) obtained from the fungal collection of the Laboratório de Fitopatologia da Universidade Estadual de Montes Claros. This isolate was selected considering the virulence tested in many assays (data not published), and 40 bacterial isolates. All of the bacterial strains tested in the present work were isolated and identified by Souza et al. (2013) and *in vitro* abilities for nitrogen fixation, phosphate solubilization and production of indol-3-acetic acid (IAA) were evaluated by Andrade et al. (2014), Gomes et al. (2017), and Matos et al. (2017) (Table I).

Microorganisms and assessments of characteristics

Fusarium oxysporum f. sp. *cubense* (*foc*) isolate 106 was growth in a Petri dish containing PDA (Potato, Dextrose and Agar) medium for seven days at 25ºC in dark conditions. 5-mm *Fusarium oxysporum* f. sp. *cubense* discs were used to perform the antibiosis tests. Spore suspensions were obtained with the addition of 20 mL of distilled water plus 1% Tween applied on colonies and by using a glass slide, the spores were placed in suspension and the number of spores was estimated in a Neubauer chamber using a Type104c light microscope (Nikon,

Table I. List of the 40 endophytic bacteria isolates identified by partial sequencing of the gene 16s rRNA, GenBank accession numbers and description of the biological capacities exhibited under *in vitro* test.

Species[I]	Isolates	GenBank access number	Abilities[II, III, IV]
Bacillus amyloliquefaciens	EB44	GU122948.1	IPS, IAP
Bacillus amyloliquefaciens	EB133	AB301022.1	BNF, IPS
Bacillus axarquienses	EB30	JF414764.1	IPS, IAP
Bacillus cereus	EB25	GU451184.1	BNF, IPS, IAP
Bacillus flexus	EB88	DQ870687.1	BNF, IPS, IAP
Bacillus licheniformis	EB49	EU366371.1	BNF, IPS, IAP
Bacillus methylotrophicus	EB26	HM209756.1	BNF, IPS, IAP
Bacillus pumilus	EB46	FJ236809.1	IPS, IAP
Bacillus pumilus	EB58	JN082265.1	IPS, IAP
Bacillus pumilus	EB15	GQ917222.1	BNF, IPS, IAP
Bacillus pumilus	EB34	JN215511.1	IPS, IAP
Bacillus pumilus	EB51	HQ218993.1	BNF, IPS
Bacillus pumilus	EB63	GQ917222.1	IPS, IAP
Bacillus pumilus	EB64	JF271873.1	BNF, IPS, IAP
Bacillus pumilus	EB169	FJ189791.1	BNF, IPS, IAP
Bacillus safensis	EB68	JN092818.1	IPS, IAP
Bacillus subtilis	EB04	AY741264.1	BNF, IPS, IAP
Bacillus subtilis	EB147	EU977724.1	IPS, IAP
Bacillus subtilis	EB84	HQ334981.1	BNF, IPS, IAP
Bacillus subtilis	EB126	HM769817.1	BNF, IPS, IAP
Bacillus subtilis	EB136	AB301012.1	BNF, IPS, IAP
Bacillus tequilensis	EB87	HM770882.1	BNF, IPS, IAP
Bacillus thuringiensis	EB24	JF947357.1	BNF, IPS, IAP
Bacillus sp.	EB71	HM461161.1	BNF, IPS, IAP
Bacillus sp.	EB47	FJ611939.1	BNF, IPS, IAP
Bacillus sp.	EB194	FJ405377.1	BNF, IPS, IAP
Bacillus sp.	EB50	HM769816.1	BNF, IPS, IAP
Bacillus sp.	EB37	JN215502.1	IPS, IAP
Bacillus sp.	COD-001	GQ340516.1	BNF, IPS, IAP
Bacillus sp.	EB81	HQ003450.1	BNF, IPS, IAP
Bacillus sp.	EB78	EU977790.1	BNF, IPS, IAP
Bacillus sp.	EB80	EU972777.1	BNF, IAP
Klebsiella pneumoniae	EB23	JN201948.1	BNF, IPS, IAP
Lysinibacillus sphaericus	EB45	JN215512.1	BNF, IPS, IAP
Lysinibacillus sp.	EB53	JN215512.1	IPS, IAP
Lysinibacillus sp.	EB60	JF906500.1	IPS, IAP
Paenibacillus sp.	EB28	EF178460.1	BNF, IPS, IAP
Paenibacillus sp.	EB144	EF178460.1	BNF, IPS, IAP
Stenotrophomonas sp.	EB38	EU931559.1	BNF, IPS, IAP
Sporolactobacillus sp.	EB127	D16282.1	BNF, IPS, IAP

Source: [I]Souza et al. (2013); [II]Andrade et al. (2014) (BNF: Biological nitrogen fixers), [III]Matos et al. (2017) (IPS: Inorganic phosphate solubilizers), [IV] Gomes et al. (2017) (IAP: Indole acetic acid producers).

Tokyo, Japan). Suspensions were adjusted to a concentration of 2 x 10^5 spores mL^{-1}.

Each bacterial isolate was cultured on 20 mL of TSA (Tryptic Soy Agar) solid medium in a Petri dish for 48 hours at 25°C. Approximately 5 mL of 0.85% NaCl was added to the colonies in order to obtain the bacterial suspensions. The concentration of bacterial cells was adjusted using a spectrophotometer to an OD540 of 1.0. These bacterial suspensions were used to evaluate the following characteristics: colony area (CAP), number of spores/cm^2 (NSP) and percentage of spores germination (%SGP) in paired culture method, percentage of spore germination in mixed solution (%SGM) and colony area (CAV), number of spores/cm^2 (NSV) and percentage of spores germination (%SGV) in volatile tests.

Antibiosis tests

The forty bacteria isolates were evaluated using a dual culture test as described by Ozaktan et al. (2015). 5-mm mycelial disks of *Fusarium oxysporum* f. sp. *cubense* from 7-day-old colonies were placed on a 9 cm diameter Petri dish containing potato dextrose medium (PDA), and almost the same time, on the opposite side of the plate bacterial suspension was streaked. The striation was performed with Drigalski handle and disposable in T format. The bacterial solution adjusted to OD540 of 1.0 absorbance. The Petri dishes were maintained in BOD in continuous darkness for seven days at 25ºC. The distance between the mycelial disks and the bacterial suspension streaked was 7 cm. In the control plates, 5-mm mycelial disk of *Fusarium oxysporum* f. sp. *cubense* from 7-day-old colonies were placed on a 9 cm diameter Petri dish containing potato dextrose medium (PDA), and on the opposite side of the plate a 0.85% saline solution was streaked. The *foc* isolate 106 colony's radius was measured by using a millimeter ruler. The radius values were converted into colony area (cm^2).

The number of spores produced by *foc* paired with bacterial isolates in each colony was quantified in a Neubauer chamber using a Type104c light microscope (Nikon, Tokyo, Japan) and the number of spores/cm^2 of mycelial area was estimated. The viability of *foc* isolate 106 spores was also determined by the germination test. In 70-mm diameter Petri dishes containing agar-water (40 g L^{-1}), a 100 µL aliquot of the spore suspension was added and incubated for 12 hours at room temperature (26°C). The percentage of spores' germination was calculated from the evaluation of 100 spores per slide using a Type104c light microscope (Nikon, Tokyo, Japan). Spores showing a germ tube length equal to or greater than the length of the spore, were considered germinated.

The direct effect of the bacterial suspension on the germination of *Fusarium oxysporum* f. sp. *cubense* spores was evaluated. Equal volumes of 1 mL of suspensions (v/v) of *foc* isolate 106 spores (10^5 spores/mL) and endophytic bacteria suspension (OD540 of 0.5 absorbance) were mixed. Approximately 100 µL of the mixed suspension (*Fusarium oxysporum* f. sp. *cubense* + bacterium) were applied on the surface of the agar-water medium in Petri dishes and incubated at room temperature (26 ºC) for 12 hours in dark conditions. The percentage of the germination was calculated from the evaluation of 100 spores per slide using a Type104c light microscope (Nikon, Tokyo, Japan).

The volatile tests were performed in Petri dishes with PDA medium. A 5-mm *Fusarium oxysporum* f. sp. *cubense* disc was deposited in the center of the Petri dishes. TSA medium was poured into the bottom of the Petri dish cover and the bacterial suspension at 1 of absorbance DO =540 nm was distributed in the form of streak with a Drigalski loop. The dishes were sealed

using plastic film and were maintained in BOD at 25ºC for seven days under dark conditions. Control treatment (control) consisted of a 5-mm *Fusarium oxysporum* f. sp. *cubense* disc deposited in the center of the Petri dishes and the TSA medium poured into the bottom of the Petri dish cover received a 0.85% saline solution streak. The colony area, number of spores/cm^2 and percentage of spores' germination were also evaluated using the same methodology previously described.

Experimental design and statistical analysis

Experiments were carried out in a completely randomized design (CRD) with 41 treatments and three replicates. Data was submitted to analysis of variance and means grouped by the Scott-Knott test at 5% probability. The "R" software version 3.5 (R Core Team 2015) was used for statistical analysis. The percentage of reduction within each variable was determined by the formula described by Mourão et al. (2003): % Reduction = [1 - (Average of Treatment / Average of Control)] * 100.

Data related to variables of *in vitro* tests were submitted to multivariate analysis, using the Principal Component Analysis (PCA) to identify which evaluated characteristics such as area of the antibiosis colony, area of the volatile colony, direct and indirect germination in the antibiosis test, germination in the volatile test, sporulation in the antibiosis and volatile test influenced the pathogen control. The dissimilarity measure used was the Euclidean distance and the hierarchical grouping method used was the UPGMA (Unweighted Pair- Group Method using Arithmetic Averages). The cutoff point of the dendrogram was chosen according to the significance of the "k" constant by the Mojena method (Mojena 1977). The analysis of the PCA was performed using the "Genes" software (Cruz 1998).

RESULTS

Table II showed a significant effect of the isolates ($p<0.05$) for all the characteristics evaluated. Normality of residues (Shapiro Wilk) and homogeneity of variances (Bartlett) tests were significant at 5% probability, demonstrating that the data follow normal distribution. The first four eigenvalues estimated for percentage of spore germination in a mixed solution, number of spores/cm^2 paired, number of spores/cm^2 volatile and colony area volatile components

Table II. Summary of the analysis of variance for the characteristics evaluated in the antibiosis tests, Janaúba, Brazil, 2019.

Variable**	Medium Square	Shapiro Wilk Test	Bartlett Test	Coefficient of variation (%)
CAP	377.9661*	0.0144	0.0000	23.77
NSP	2940.4283*	0.0002	0.0001	28.62
%SGP	919.2483*	0.0000	0.0000	1.59
%SGM	2480.1655*	0.0002	0.0000	7.07
CAV	134.346*	0.0315	0.0000	17.60
NSV	708.8297*	0.0000	0.0000	36.24
%SGV	1686.2012*	0.0000	0.0000	5.91

***Significant at 5% probability ($p<0.05$) by the F test. **CAP: colony area paired, NSP: number of spores/cm^2 paired, %SGP: percentage of spores germination paired, %SGM: percentage of spore germination in mixed solution, CAV: colony area volatile, NSV: number of spores/cm^2 volatile and %SGV: percentage of spores germination volatile.**

represented about 86.10% of the total variance observed (Table III).

The percentage of spore germination (%SGM) component in the presence of bacterial cells was the most important for the study, as it was derived from the largest eigenvalue and explains around 29.88% of the total variation. The second most important component is the number of spores/cm^2 in pairing, derived from the second largest eigenvalue and explains around 23.05%. Components associated with sporulation percentages and colony area in volatiles accumulated a percentage of cumulative explained variance of 33.17% (Table III).

Fifteen different groups were observed and 67.5% of isolates were significantly different from the control treatment (Figure 1). The mixed solution containing bacterial cells from isolate EB37 promoted a reduction of 96% in the germination of *Fusarium oxysporum* f. sp. *cubense* spores (Figure 1). The isolates EB28, EB23 and EB127 reduced over 70% of spores' germination.

Five different groups in pairing assay and three groups in the volatile test on *Fusarium oxysporum* f. sp. *cubense* sporulation were observed (Figure 2a and 3). The number of spores/cm^2 were significantly reduced for both tests: pairing and volatile. Sixteen bacterial isolates (Figure 2d) in the pairing test presented a variation from 12.22 to 36.67 spores/cm^2 including the isolate EB37 with 84,6% the reduction. The control treatment (Figure 2a and b) and isolates EB34, EB63, EB144 presented 76.57 ⊦ 89.67 spores/cm^2 (Figure 2a). Three isolates EB04, EB194 and EB53 produced significant increase of the magnitude of 47,9; 36,6 and 117,1% in the number of spores/cm^2 (Figure 2a).

The number of spores/cm^2 in the volatile test were reduced significantly by 87.5% of bacterial isolates (Figure 3). Isolates EB37, EB15 and COD-001 reduced between 65 and 75% of the spore production. However, the isolate EB194 stimulated the production of spores in 117% in the volatile test (Figure 3). Two distinct groups were observed for colony area (CAV) in volatile test (Figure 4a). Twenty-three isolates showed significant reductions and two of them, EB136 and EB51 (Figure 4d and 4e), reduced the colony area of the *F. oxysporum* f. sp. *cubense* up to 54.62%.

The UPMGA clustering method based on Euclidean distance divided the 40 endophytic

Table III. Principal components, eigenvalues, percentage of explained variance and percentage of cumulative explained variance for characteristics evaluated in the antibiosis tests, Janaúba, Brazil, 2019.

Principal component	Eigenvalues	Explained variance (%)	Cumulative explained variance (%)	Recommendation
%SGM	2.09	29.88	29.88	-
NSP	1.63	23.05	52.93	-
NSV	1.41	20.19	73.12	-
CAV	0.90	12.98	86.10	-
%SGP	0.63	9.10	95.20	Discard
%SGV	0.21	3.05	98.25	Discard
CAP	0.12	1.75	100	Discard
Pillai Test = 1.411446e^{-104}				

CAP: colony area paired, NSP: number of spores/cm^2 paired, %SGP: percentage of spores germination paired, %SGM: percentage of spore germination in mixed solution, CAV: colony area volatile, NSV: number of spores/cm^2 volatile and %SGV: percentage of spores germination volatile.

bacteria isolates into eight groups. Using an 80% cutoff point, all groups were homogeneous and distinct from each other (Figure 5). The dissimilarity analysis converted to a dendrogram showing a coefficient of correlation of 80%, which means that the dendrogram is similar in 80% of dissimilarity matrices generated by the UPGMA method. Groups II, IV and VII consisted exclusively of one treatment: EB53, control and EB28, respectively. Groups I and V were composed of two isolates each. The largest group was VI which comprised 42.5% of all bacterial isolates and these were effective in controlling at least one of the characteristics evaluated (Figures 1, 2, 3 and 4) (Figure 5).

DISCUSSION

The results of the present study indicated that the *foc* 106 was affected in its life cycle under *in vitro* conditions. The effects of antibiosis tests were observed in reducing the number of spores/cm^2, colony area and percentage of spore germination and/or in the initial formation of the germ tube in a mixed solution, pairing and/or volatile tests.

Three characteristics (%SGM, NSP and NSV) contributed to a large extent in the study with more than 70% of cumulative explained variance. Principal component analysis is used as a criterion to judge the importance of the selected variables; the greater the weight of the linear combination of the variables of the first principal component, the greater the statistical importance of that combination (Santos et al. 2019). Application of this tool allows principal variables to be distinguished, making the evaluation more efficient and reducing the experimental work and costs (Teixeira et al. 2013).

Bacillus sp. (EB 37), *Bacillus flexus* (EB 88), and *Sporolactobacillus* sp. (EB 127) isolates inhibited more than 70% of the *Fusarium oxysporum* f. sp. *cubense* spores' germination in the mixed solution test. Special attention should be paid to EB37 isolate, which inhibited

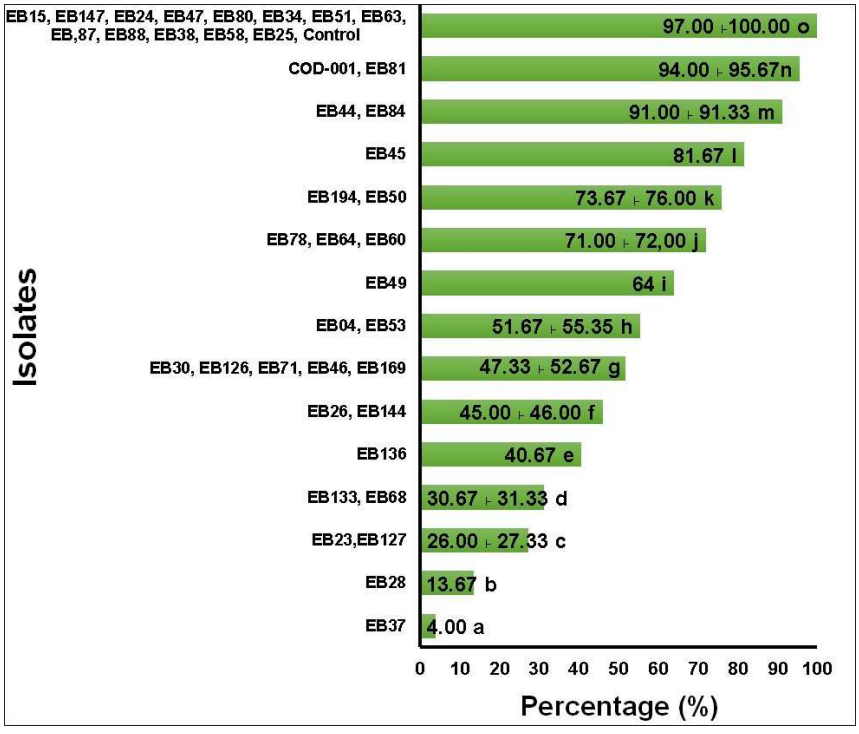

Figure 1. Percentage of *Fusarium oxysporum* f. sp. *cubense* isolate 106, spores' germination in a mixed solution (*foc* 106 + bacterial isolates) (%SGM) with different bacterial isolates, Janaúba, Brazil, 2019. Averages followed by the same letter in the column do not differ by the Scott Knott's test at 5% probability. Coefficient of variation 7.07%.

96% of the *foc* 106 spores' germination and it was the most effective among all isolates evaluated. Biological control of plant disease by *Bacillus* species involves several mechanisms such as production of antifungal metabolites (bacillomycin, iturin, fengycin), cell wall-degrading enzymes (amylases, proteases, cellulase, pectinase, glucanase), and host resistance induction (Arrebola et al. 2010). In addition to this antagonic capacity to *foc* 106 previous studies have reported the biological capacity of *Bacillus* sp. (EB 37) to solubilize inorganic phosphate and biosynthesize indole acetic acid (Matos et al. 2017, Gomes et al. 2017). To the best of our knowledge, this represent the first report on the ability of this strain to control under *in vitro* conditions the spore germination of *Fusarium oxysporum* f. sp. *cubense* (*foc*) isolate 106.

Although in the present work none experimental evidence was built in order to identify secondary metabolites associated to inhibiting the emission and/or elongation of the germ tube such as iturins, fengycin and siderophores, the present results pointed an effective action of the isolate, *Bacillus* sp. (EB 37) on *foc* 106 and additional studies must be performed in order to elucidate these

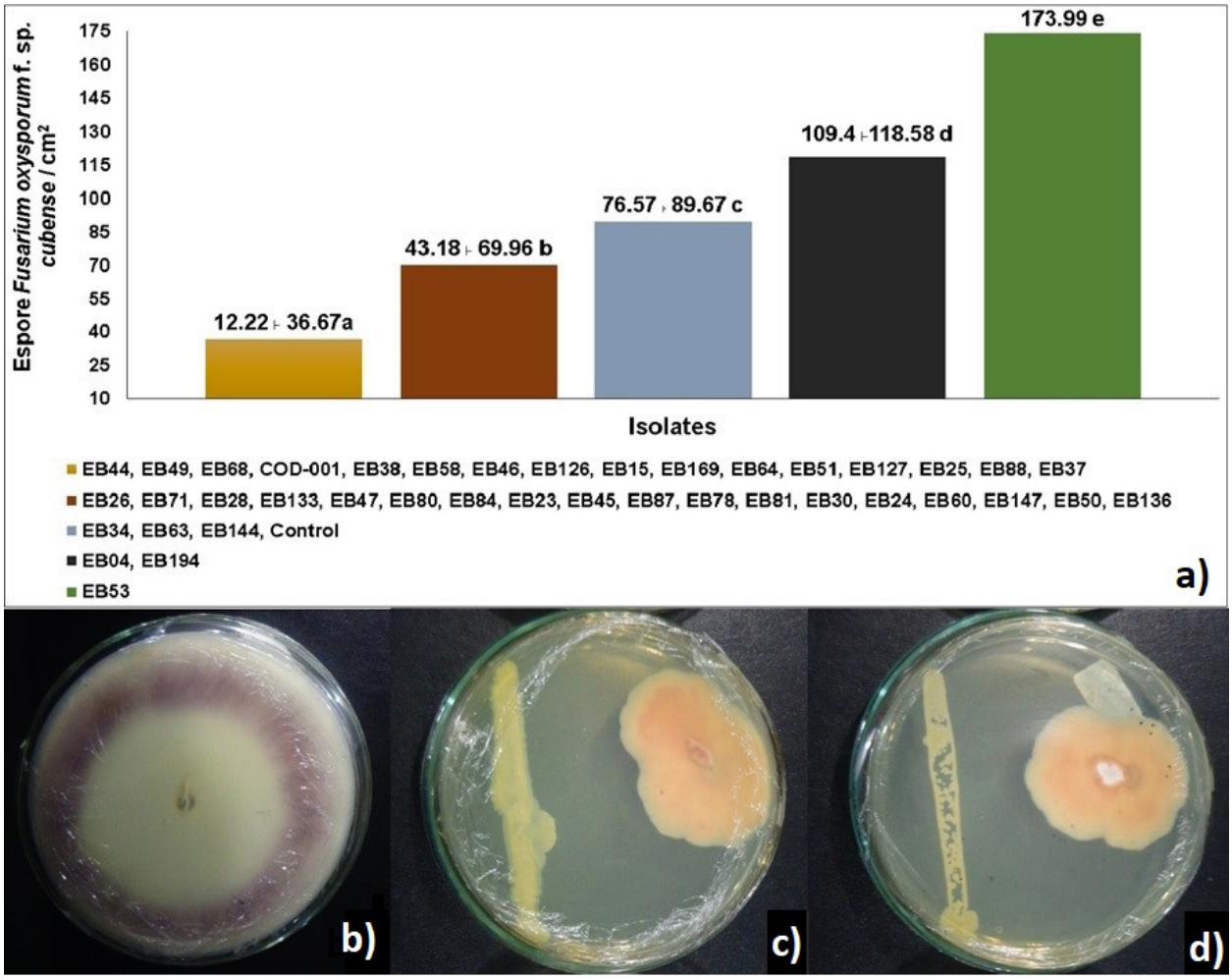

Figure 2. a) Number of *Fusarium oxysporum* f. sp. *cubense* spores/cm² in pairing tests (NSP) with different bacterial isolates, Janaúba, Brazil, 2019. **b)** Control, **c)** Isolate EB136 and **d)** EB37. Averages followed by the same letter in the column do not differ by the Scott Knott's test at 5% probability. Coefficient of variation 28.62%.

mechanisms (Lee et al. 2017, Bruisson et al. 2019). Bacteria of the genus *Bacillus* have great ability to produce many antifungal components. The siderophores have been very well documented as an efficient organic compound that bind to Fe^{3+} ions and transport them into its cells, making the Fe^{3+} unavailable or reduced for other microorganisms (Ahmed & Holmström 2014). In conditions of deficiency of this ion, the elongation of the germ tube can be affected and paralyze the microorganisms' growth (Chan et al. 2003).

In biological control, it is desirable that the agent reduces the new life cycles of the phytopathogen, acting on the reduction of propagules. The isolates: COD-001, EB15, EB38, EB25, EB51, EB68, EB58, EB37, EB127, EB126 and EB88 deserve attention due to their ability in reducing the number of spores/cm^2 in pairing and volatile tests. The isolates EB37 was able to reduce 74.4 and 84.7%, respectively. Some authors reported that in the field, these effects have the potential of reducing the disease, by decreasing the phytopathogen hyphae, preventing the establishment of phytopathogens and by inhibiting their phytopathogenic activity after colonization in the plant (Palazzini et al. 2016, Figueroa-López et al. 2016).

We also observed that four isolates EB04, EB144, EB194 and EB53 incremented the number of spores/cm^2 in antibiosis test in 117.1% in interaction with the isolate EB53. It is possible that the production of fusaric acid has affected the performance of bacteria isolates. *Fusarium oxysporum* f. sp. *cubense* produces fusaric acid (FA) and it is involved in virulence on banana plants (Ding et al. 2018), enhancing the capacity of the pathogen in avoiding damaging mechanisms, such as protection from oxidative stress (Reverberi et al. 2010) and from microbial competitors in their natural habitat (Palumbo et al. 2008).

Some isolates beyond the reduction the number of spores/cm^2 also reduced the colony area in the volatile test: EB44, EB68, COD-001, EB38, EB46, EB51, EB127, EB169, EB25, EB127, EB126, and EB88. The production of volatile compounds is an interesting attribute of a microorganism.

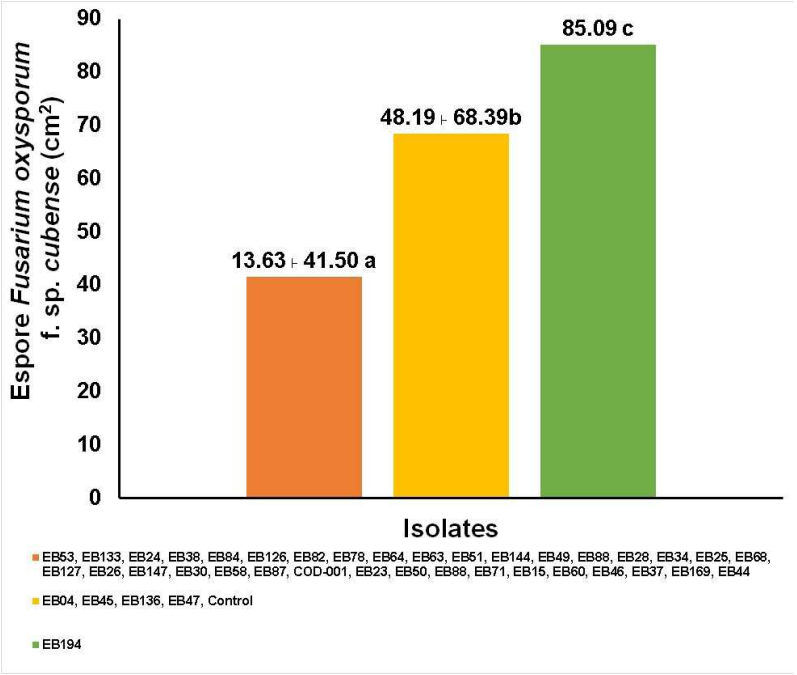

Figure 3. Number of *Fusarium oxysporum* f. sp. *cubense* spores/cm^2 in the volatile tests (NSV) with different bacterial isolates, Janaúba, Brazil, 2019. Averages followed by the same letter in the column do not differ by the Scott Knott's test at 5% probability. Coefficient of variation 36.24%.

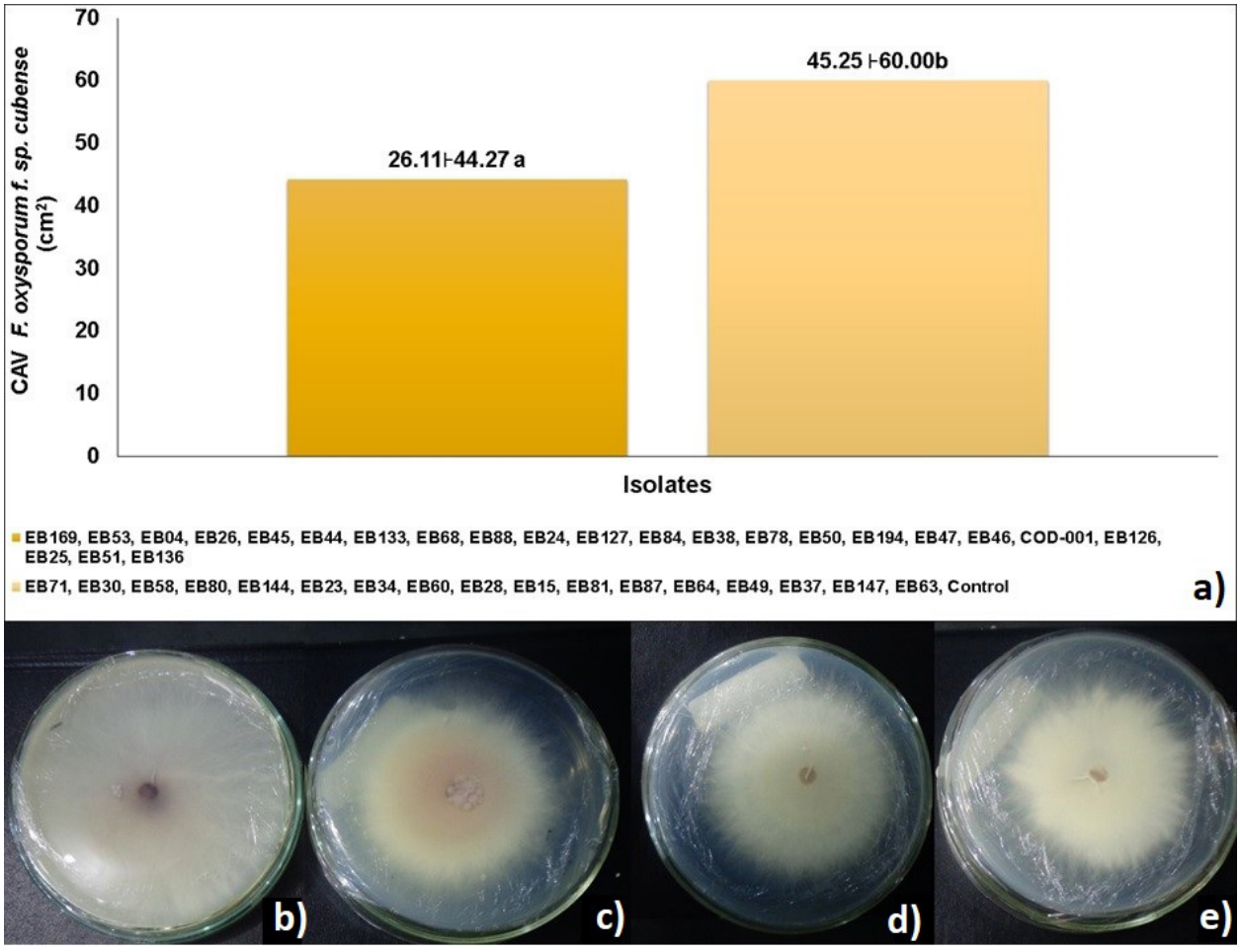

Figure 4. a) Colony area of *F. oxysporum* f. sp. *cubense* in volatile test (CAV) with different bacterial isolates, b) Control, c) EB126, d) EB51 and e) EB136. Janaúba, Brazil, 2019. Averages followed by the same letter in the column do not differ by the Scott Knott's test at 5% probability. Coefficient of variation 17.60%.

These molecules are carbon-based solids and liquids that enter the gas phase vaporizing at 0.01kPa at a temperature of approximately 20ºC (Pagans et al. 2006). These compounds are primary and secondary derivates of microbial metabolism and are able to diffuse both in the atmosphere and in the soil, so they can reach the phytopathogen at more distant points and cause its inhibition (Korpi et al. 2009, Morath et al. 2012). Yuan et al. (2012) verified in their experiments that *Bacillus* spp. produces volatile compounds capable of inhibiting mycelial *Fusarium oxysporum* f. sp. *cubense* growth.

A criterion that quantifies the distance among individuals is understood as a measure of dissimilarity and therefore, the lower the values of these averages, the more similar the individuals. The UPMGA clustering method based on Euclidean distance divided the 40 endophytic bacteria isolates and the treatment control. The group VIII presented highest dissimilarity values compared to group IV (control). This group is composed by 12 isolates and among them the isolate EB 37. Considering the potential of the isolate EB 37 (*Bacillus* sp.) showed in the present work additional studies from the genomic perspective must be considered. These studies

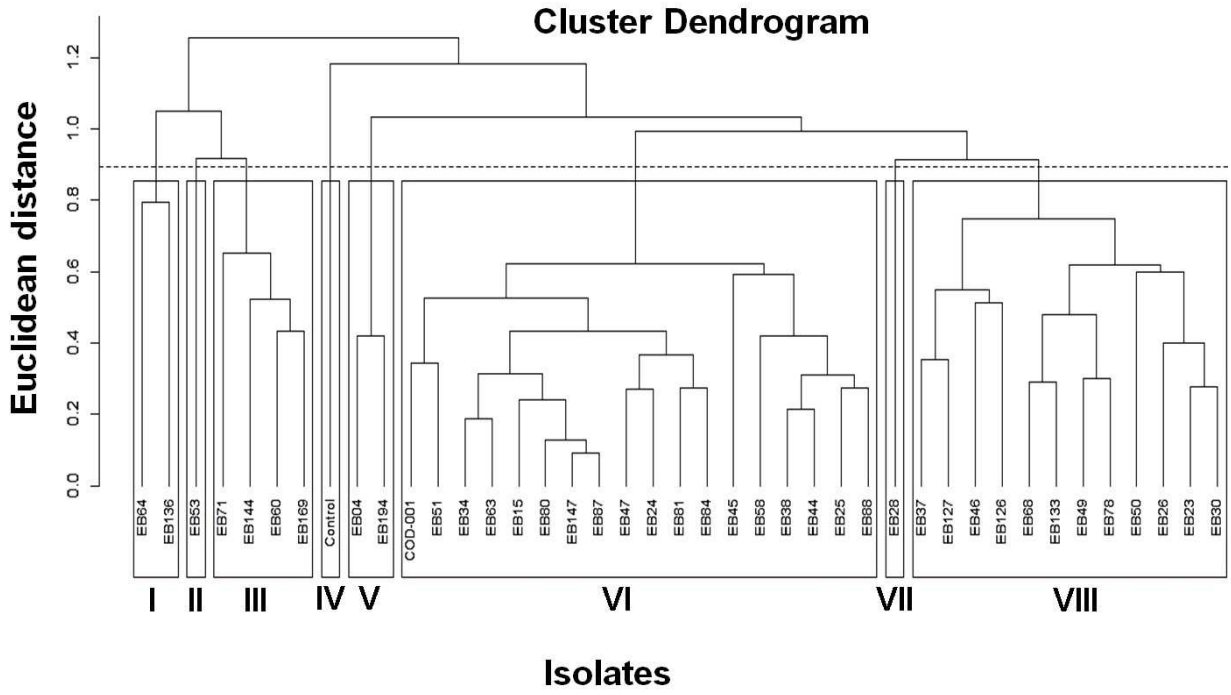

Figure 5. Dendrogram of genetic dissimilarity constructed with the Jaccard index and UPGMA clustering method, showing clusters formed among the 40 endophytic bacteria isolates. I, II, III, IV, V, VI, VII and VIII are the number of groups performed according to Mojena (1977).

are important to establish the safety and efficiency of this microorganism in industrial microbiology.

The comprehension of the mechanisms of beneficial bacteria is particularly important to develop commercially efficient and successful biocontrol strategies against plant pathogens (Raza et al. 2013). Among the strategies available are the isolation, the identification and the characterization of native and autochthonous microorganisms are very recommended and effective in biological control (Ahmad et al. 2018). The introduction of a large quantity of "exotic" microorganisms may disrupt a local ecosystem and produce ecological impacts on the rhizosphere microbiota (Ambrosini et al. 2016).

All endophytic bacteria evaluated in the present study were isolated from roots of banana cultivar Prata Anã and therefore are considered autochthonous. The autochthonous and/or native microorganisms are preferred for many reasons: (1) superior performance for controlling diseases, for promoting plant-growth and increasing yield in its original host; (2) high affinity with the root system and ability to establish endophytic interaction; (3) well suited for edaphic and climatological conditions from a specific region; and (4) the co-existence for many years with the natural soil microbiota should provide native and autochthonous microorganisms with competitive advantages compared to exotic species survival.

CONCLUSIONS

All isolates reduced one or more biological events in the pathogen cycle. The variable that best explains the response of bacterial isolates to *Fusarium oxysporum* f. sp. *cubense* is germination in the presence of bacterial cells. *Bacillus* spp., isolate EB37 was the most effective

in reducing *Fusarium oxysporum* f. sp. *cubense* spores' germination in the mixed solution test and was effective in reducing the number of spores/cm^2 in paired and volatile tests.

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MARIA J. MARTINS[1]
https://orcid.org/0000-0003-1911-5679

ADELICA A. XAVIER[1]
https://orcid.org/0000-0001-9814-4587

ISABELLE C. CARDOSO[1]
https://orcid.org/0000-0002-4843-4524

DANIELA F. SILVEIRA[1]
https://orcid.org/0000-0002-3892-9160

REGINA C.F. RIBEIRO[1]
https://orcid.org/0000-0001-5138-8402

SAMY PIMENTA[2]
https://orcid.org/0000-0002-9411-9381

SILVIA NIETSCHE[3]
https://orcid.org/0000-0002-8188-3057

[1]Universidade Estadual de Montes Claros, Laboratório de Microbiologia e Nematologia do Departamento de Ciências Agrárias, Av. Reinaldo Viana, 2630, 39440-000 Janaúba, MG, Brazil

[2]Universidade Estadual de Montes Claros, Laboratório de Biotecnologia do Departamento de Ciências Agrárias, Av. Reinaldo Viana, 2630, 39440-000 Janaúba, MG, Brazil

[3]Universidade Federal de Minas Gerais, Laboratório de Microscopia, Microanálises e Biologia do Instituto de Ciências Agrárias, Av. Universitária, 1000, 39404-547 Montes Claros, MG, Brazil

Correspondence to: **Silvia Nietsche**
E-mail: silvia.nietsche@gmail.com

Author contributions

MJM and AAX designed the experiments. MJM, ICC and DFS performed the experiments with assistance from AAX and RCFR. MJM, SP, and AAX, performed the statistical analysis of the data. MJM, AAX, SN and RCFR discussed the results. AAX and SN coordinated and merged the authors' individual contributions. All authors read, revised and approved the submitted version.