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Artículo

Antifungal activity of *Bacillus amyloliquefaciens* against *Fusarium oxysporum* f. sp. cubense race 1 [1]

Actividad antifúngica de *Bacillus amyloliquefaciens* contra *Fusarium oxysporum* f. sp. cubense raza 1

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Abstract:

Introduction. Due to the absence of totally effective either economically viable chemical agents for the control of Fusarium wilt, the use of antagonistic microorganisms is of great interest since it could represent a more economically and ecologically sustainable alternative. **Objective.** To analyze the antifungal effect of the *Bacillus amyloliquefaciens* CCIBP-A5 strain against *Fusarium oxysporum*. **Materials and methods.** The work was carried out in the Laboratory of Applied Microbiology of the Instituto de Biotecnología de las Plantas, Cuba, between September 2017 and June, 2018. The in vitro and in vivo antifungal activity of its culture filtrate and cell against *F. oxysporum* has been assayed. **Results.** The results indicated that the metabolites present in the culture filtrate of *B. amyloliquefaciens* CCIBP-A5 significantly influenced the growth and morphology of the mycelium and the conidia. They also caused oxidative damage to the lipid molecules of *F. oxysporum*. In addition, this strain showed inhibitory effects on the development of the disease under controlled conditions. These aspects are key when selecting a bacterial candidate as a biological control agent. **Conclusions.** The results showed that the *B. amyloliquefaciens* CCIBP-A5 strain, isolated from *Musa* sp., had an *in vitro* antifungal effect against the vegetative and reproductive structures of Foc race 1 as well as on the *Musa* spp.-*F. oxysporum* interaction. This strain is suggested for the development of a bioproduct for Fusarium wilt management.

Keywords: antagonist, biological control, Fusarium wilt, metabolites.

Resumen:

Introdución. Debido a la ausencia de agentes químicos totalmente efectivos o económicamente viables para el control del marchitamiento por Fusarium, el uso de microorganismos antagonistas es de gran interés ya que podría representar una alternativa más sostenible desde el punto de vista económico y ecológico. **Objetivo.** Analizar del efecto antifúngico de la cepa Bacillus

amyloliquefaciens CCIBP-A5 contra *Fusarium oxysporum*. **Materiales y métodos.** El trabajo se realizó en el laboratorio de Microbiología Aplicada del Instituto de Biotecnología de las Plantas, en Cuba, entre septiembre 2017 y junio 2018. Se analizó la actividad antifúngica *in vitro* e *in vivo* de las células y su filtrado de cultivo frente a F. oxysporum. Resultados. Los metabolitos presentes en el filtrado de cultivo de *B. amyloliquefaciens* CCIBP-A5 influyeron significativamente en el crecimiento y la morfología del micelio y los conidios. También causaron daño oxidativo en las moléculas de lípidos de *F. oxysporum*. Además, esta cepa mostró efectos inhibitorios sobre el desarrollo del patógeno en condiciones controladas. Estos aspectos son clave cuando se selecciona un candidato bacteriano como agente de control biológico. **Conclusiones.** Los resultados mostraron que la cepa de *B. amyloliquefaciens* CCIBP-A5, aislada de *Musa* sp., tuvo un efecto antifúngico *in vitro* contra las estructuras vegetativas y de reproducción de Foc raza 1, así como en la interacción *Musa* spp. - *F. oxysporum*. Esta cepa se propone para el desarrollo de un bioproducto para el manejo de la marchitez por Fusarium.

Palabras clave: antagonista, control biológico, marchitez por Fusarium, metabolitos.

Introduction

The production of bananas and plantain is affected by numerous diseases, among which the Fusarium wilt, caused by the fungus *Fusarium oxysporum* Schlecht f. sp. *cubense* (E.F. Sm.) Snyder and Hansen could be highlighted. This disease is considered the tenth most significant in the history of agriculture due to the large economic losses it has caused (Dita-Rodríguez et al., 2013) bringing about the most destructive effects on the plant (Anthony et al., 2017).

Fusarium oxysporum f. sp. *cubense* is genetically and pathogenically diverse since it has more than 20 vegetative compatibility groups (VCG's), distributed in four pathogenic races (race 1, 2, 3 and 4) (Pérez-Vicente & Dita, 2014; Ploetz, 2015a). Its geographic distribution is very wide and causes great economic losses in Latin America, Africa, and a large part of Asia (Stover & Simmonds, 1987).

Due to the absence of totally effective either economically viable chemical agents for the control of the disease (Ploetz, 2015b), producers have integrated different preventive methods obtaining certain success in the cultivation of the different cultivars. Among the most widely used methods are shifting cultivation, annual staggered plantings, the search for pathogen-free land (Pérez-Vicente, 2016), crop rotation (Huang et al., 2012) and the use of certified plants as pathogen-free, obtained from tissue culture (Dita-Rodríguez et al., 2013). However, these actions only allow the cultivation of susceptible cultivars for short periods, since the fungus reappears in the soil to devastate the plantations (Pérez-Vicente, 2016). Given this scenario, the use of antagonistic microorganisms is of great interest since it could represent a more economically and ecologically sustainable alternative.

To evaluate the fungal growth, different methods have been used, among which stand out: measurement of the radial growth of the colonies (Hernández-Castillo et al., 2008), determination of the dry mass of the mycelium and the reading of turbidity by absorbance (Broekaert et al., 1990). The last one has been used in studies by Cruz-Martín et al. (2013) to quantify the *in vitro* growth of *Pseudocercospora fijiensis* Morelet.

It has been shown that certain bacterial strains under controlled conditions suppress the growth of *F. oxysorum* (Ho et al., 2015; Sekhar & Thomas, 2015; Simonetti et al., 2018; Zacky & Tiny, 2013). Within these bacterial groups several strains of the genus *Bacillus* have been described (Wiyono & Widono, 2013; Xue et al., 2015). This genus, in particular, has been widely studied as a biological control agent due to its multiple mechanisms of antagonistic action such as antibiosis, competition, and the induction of systemic resistance in plants (Chowdhury et al., 2015; Fan et al., 2017). In addition, it is distinguished from other bacterial genera such as *Pseudomonas* by forming endospores that allow them to survive for long periods under unfavorable environmental conditions (Radhakrishnan et al., 2017). These characteristics make the biofertilizers based on *Bacillus* spp. more active, since it increases the viability of the cells within the formulations (Gang et al., 2013).

Various strains of *B. amyloliquefaciens* have been previously studied for their antifungal properties against a large number of phytopathogenic fungi. This bacterial species has been widely recognized for combining

several of its antifungal mechanisms, which promotes efficient biocontrol (Liu et al., 2017). Other studies have shown that the mycelial growth of various phytopathogenic fungi such as: *Botrytis cinerea*, *Ralstonia solanacearum*, and *Endothia parasitica* (Murrill) Barr, were inhibited by compounds produced by strains of *B. amyloliquefaciens* (Raza et al., 2016). In a recent report, the isolate CCIBP-A5, out of 17 bacillus strains, was selected for achieving the highest percentage values of radial growth inhibition activity against *F. oxysporum* (Leyva et al., 2017). Also, this strain was able to produce volatile and diffused antifungal metabolite on *in vitro* condition. Metabolites with antifungal activity produced by *Bacillus* spp. present diverse chemical structures which determine their biological activity (Bacon & Hinton, 2011). Antibiotics of lipopeptide nature constitute one of the most important (Gond et al., 2015). However, these compounds can stimulate the formation of chlamydospores in several filamentous fungi at certain concentrations below the inhibitory minimum (Li et al., 2012). Other authors indicated that the antifungal effect of several strains of *Bacillus* against *F. oxysporum* f. sp. *lycopersici*, including a strain of *B. amyloliquefaciens*, was mediated by the action of lipopeptide compounds and also by the action of hydrolytic enzymes such as chitinases (Abdallah et al. 2017). The current study was carried out with the objective to the analyses of antifungal effect of *Bacillus amyloliquefaciens* CCIBP-A5 strain against *Fusarium oxysporum*.

Material and methods

The research was made in Laboratory of Applied Microbiology of the Instituto de Biotecnologia de las Plantas, Cuba, between September 2017 and June 2018.

The strains CCIBP-A5 was isolated from banana phyllosphere, and phenotypic characterization by physiological and biochemical tests was performed according to standard methods (Krieg and Holt, 1984). To confirm their identification, 16S rDNA sequence homology analysis was undertaken. The 16S rDNA gene of CCIBP-A5 was amplified using the forward primer 27F (AGAGTTTGATCMTGGCTCAG) and the reverse primer 907R (CCGTCAATTCMTTTRAGTTT) (Lane, 1991). Sequence comparison to standard databases was performed using BLAST through the NCBI server.

For culture filtrated (CF) preparation, an overnight grown culture of strain CCIBP-A5 was centrifuged (10 min at 4 °C and 12 000 g) and cells were adjusted to an OD 600 nm of 0.1 in deionized water. 1 mL of bacterial suspension was inoculated in 100 ml nutrient broth (NB) culture media (Fluka, Germany). The strain treated for 72 h at 30 °C and 120 rpm in an orbital shaker (Gerhardt), centrifuged at 12 000 g for 10 min at 4 °C and filtered with a 0.2 µm membrane (Millipore).

The pathogenic fungus *F. oxysporum* strain CCa 1.1 VCG (Foc) was donate by Instituto Nacional de Sanidad Vegetal (INISAV), Havana, Cuba. This fungus was grown in potato dextrose broth (PDB) culture media (BioCen, Cuba) for 7 days at 28 °C with a rotatory shaker at 120 rpm. The fungi culture was passed through a 100 µm sieve. The micelial suspension (MS) was prepared by mixing the mycelium in Ultra-Turrax T25 homogenizer (Rose Scientific Ltd., Canada) for 1 mm and the concentration was adjusted using a hematocytometer (5×10^6 fragment ml^{-1}). The supernatant was centrifuged (10 min at 4 °C and 8000 g) (Eppendorf Centrifuge 5810 R), and conidial suspension were adjusted to 106 conidia ml^{-1} with sterile water.

In order to determine the antifungal activities of diffused compounds, the effect of CF on *Foc* vegetatives and reproductives structure were evaluated.

The culture filtrate (CF) was diluted at 1:10 and 1:100 (v/v) in MS and incubated for 120 h at 28 °C. The incubation time of the conidial suspension with the CF was decided from previous studies carried out by Abdallah et al. (2015). The researchers showed that in the *Bacillus* strains evaluated, three and four days are the optimal incubation duration for the production of more effective antifungal metabolites. In addition, they reported that the extracellular metabolites found in the CF of these strains had significant bioactivity against *F. oxysporum* f. sp. *lycopersici* about 50-100 °C.

The control treatment contained NB inoculated with MS. Fungal growth was determined by absorbance reading according to Cruz-Martín et al. (2013) using 96 wells microtiter plates. In addition, mycelia were observed under the optical microscope (400 x, 1000 x) and described. The experiment was repeated twice using eight replies per treatment. Similar assay was conducted to evaluate the effect of CF on conidial germination. Instead of MS, conidial suspensions were used.

The effect of CF in oxidative stress was assessed by determination of lipid peroxidation. The samples were obtained by inoculating the MS in a flask with PDB medium and diluted in CF (1:10). The flasks were incubated for 48 h at 28 °C in the dark and mycelia were separated by centrifugation for 5 min at 4 °C and 10 000 g. The extent of lipid peroxidation [malondialdehyde (MDA) as principal product] was estimated using the thiobarbituric acid assay described by Choi et al. (1996).

In order to validate the potential of bacterial strain, its effect on the development of the disease under controlled conditions was evaluated. Following the protocol described by Cong et al. (2017) with some modifications, the plants were placed in 1 L capacity containers with 250 mL of Hoagland solution. Three-month acclimatized Gros Michel (AAA) plants (five per treatment) were used and the following treatments were included: Plants in Hoagland solution (T1), Plants in Hoagland solution with a conidial suspension (T2), and a root immersion was performed in a bacterial suspension of strain CCIBP-A5 for 30 min and subsequently placed in Hoagland solution with conidial suspension (T3).

The plants were placed in the growth chamber (FITOTRON) where the environmental conditions were adjusted to 28 ºC day / 25 ºC night, relative humidity of 50-70 % and a photoperiod of 16 light hours, 8 dark hours. In addition, 3 daily irrigations were performed by manual spraying. The evaluation of the experiment was carried out after 22 days and consisted in the visual observation of the typical symptoms of Fusarium wilt in the plants according to the scale proposed by Dita et al. (2014). This scale has been used for evaluation of Fusarium wilt of banana in greenhouse conditions based on external and internal symptoms. Classes for external symptoms were: 1: no symptoms, 2: initial yellowing mainly in the lower leaves, 3: yellowing of all the lower leaves with some discoloration of younger leaves; 4, all leaves with intense yellowing, 5: plant dead. Internal symptoms were: 1: No symptoms; 2: initial rhizome discoloration; 3: slight rhizome discoloration along the whole vascular system, 4: rhizome with most of the internal tissues showing necrosis, 5: rhizome totally necrotic.

Results

The strain CCIBP-A5 was molecularly corroborated as *Bacillus amyloliquefaciens*. The partial 16S rDNA sequences determined in this study revealed 99 % homology with *Bacillus amyloliquefaciens* subsp. *plantarum* strain Ht1-1 in the GenBank (*JF899275.1*).

Effect of culture filtrate on mycelium

The methodology used showed that the compounds present in the culture filtrate (CF) of the selected strain significantly inhibited the growth of *Foc* (Figure 1A). When the CF was used in a ratio of 1:10 and 1:100, the mycelial growth of *Foc* was inhibited by 25 and 23.46 %, respectively, in relation to the control. These results indicated that the compounds present in the CF had an antifungal effect, even at very low concentrations. As a result of microscopic observation, it was found that the CF of strain CCIBP-A5 caused changes in the mycelial morphology of Foc (Figure 1B). Deformities and swelling were observed in the hyphae, abundant vacuolization in the protoplasm and the formation of chlamydospores.

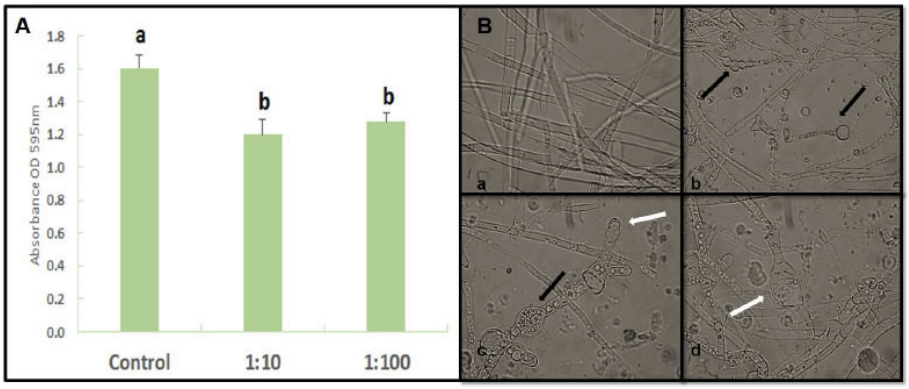

FIGURE 1

Effect of culture filtrate (CF) of *B. amyloliquefaciens* CCIBP-A5 on in vitro growth of *F. oxysporum* race 1. Laboratory of Applied Microbiology, Instituto de Biotecnologia de las Plantas, Cuba, 2018. A) *F. oxysporum* mycelial suspension in the presence of CF at a ratio of 1:10 and 1:100 (v/v) after five days of incubation. The different letters in the columns indicate significant differences ($p<0.05$) for the Kruskal-Wallis/U test of Mann Whitney (n=8) and the vertical bars indicate standard deviation. B) Effect of CF of *B. amyloliquefaciens* CCIBP-A5 in a ratio of 1:10 (v/v) on the morphology of *F. oxysporum* mycelium after five days of incubation. Control (*F. oxysporum* mycelial suspension without CF) (a). The white arrows indicate deformations and abundant vacuolization in the hyphae (c and d) and the black arrows the formation of chlamydospores (b and c). Magnification: 1000X (a, c, and d). Magnification: 400X (b).

Figura 1. Efecto del filtrado de cultivo (CF) de *B. amyloliquefaciens* CCIBP-A5 en el crecimiento *in vitro* de *F. oxysporum* raza 1. Laboratorio de Microbiología Aplicada, Instituto de Biotecnología de las Plantas, Cuba, 2018. A) Suspensión micelial de *F. oxysporum* en precencia del CF a razón de 1:10 y 1:100 (v/v) después de cinco días de incubación Letras diferentes en las columnas indican diferencias significativas ($p<0,05$) por la prueba Kruskal-Wallis/U de Mann Whitney (n=8) y las barras verticales indican la desviación estándar. B) Effecto del CF de *B. amyloliquefaciens* CCIBP-A5 en relación 1:10 (v/v) en la morfologia del micelio de *F. oxysporum* después de cinco días de incubación. Control (Suspensión micelial de *F. oxysporum* sin CF) (a). Las flechas blancas indican deformaciones y abundante vacuolización en las hifas (c y d) y las flechas negras formación de clamidosporas (b y c). Aumento: 1000X (a, c y d). Aumento: 400X (b).

Effect of culture filtrate on conidia

In this bioassay, the absorbance values obtained from the conidial suspension in the presence of culture filtrate (CF) of CCIBP-A5, decreased significantly in respect to the control (Figure 2A).

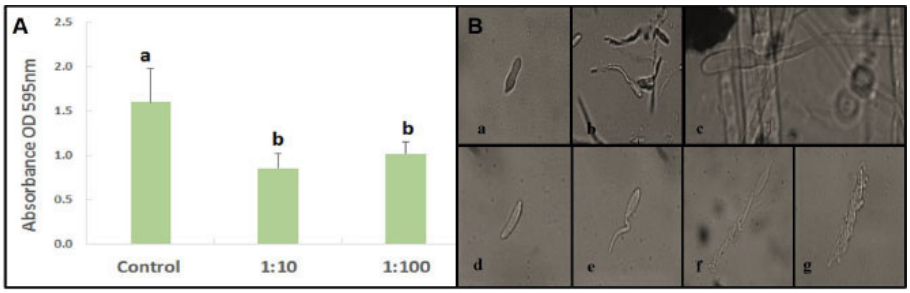

FIGURE 2

Effect of culture filtering (CF) of *B. amyloliquefaciens* CCIBP-A5 on the growth and germination of *F. oxysporum* race 1 conidia. Laboratory of Applied Microbiology, Instituto de Biotecnologia de las Plantas, Cuba, 2018. A) *In vitro* growth of *F. oxysporum* from a conidial suspension in presence of CF of *B. amyloliquefaciens* CCIBP-A5 in a ratio of 1:10 and 1:100 (v/v) after five days of incubation. The different letters in the columns indicate significant differences ($p<0.05$) by the Mann Whitney Kruskal-Wallis/U test of Mann Whitney (n=8) and the vertical bars indicate standard deviation. B) Conidia morphology of *F. oxysporum* on different stages of germination in the presence of CF in a ratio of 1:10 (v/v) after five days of incubation (d, e, f and g). Conidia morphology without the presence of CF (a, b and c). Magnification: 400X (a, b, d, e, f and g). Magnification: 1000X (c).

Figura 2. Efecto del filtrado de cultivo (CF) de *B. amyloliquefaciens* CCIBP-A5 en el crecimiento y la germinación de conidios de *F. oxysporum* raza 1. Laboratorio de Microbiología Aplicada, Instituto de Biotecnología de las Plantas, Cuba, 2018. A) Crecimiento *in vitro* de *F. oxysporum* a partir de suspensiones conidiales en presencia de CF de *B. amyloliquefaciens* CCIBP-A5 a razón de 1:10 y 1:100 (v/v) después de cinco días de incubación. Letras diferentes en las columnas indican diferencias significativas ($p<0,05$) por la prueba Kruskal-Wallis/U de Mann Whitney (n=8) y las barras verticales indican la desviación estándar. B) Morfología de conidios de *F. oxysporum* en diferentes estadios de germinación en presencia del CF a razón de 1:10 (v/v) después de cinco días de incubación (d, e, f y g). Morfología de conidios sin la presencia del CF (a, b y c). Aumento: 400X (a, b, d, e, f y g). Aumento: 1000X (c).

With the use of CF in a ratio of 1:10 and 1:100, an inhibition of Foc was observed in 49 and 36.9 % in relation to the control, respectively. The results of the microscopic observations let to verify the effects of the CF of the strain CCIBP-A5 on the germination of the conidia. The CF caused changes in the morphology of *Foc* conidia during different stages of germination (Figure 2B).

Effect of culture filtrate on the cytoplasmic membrane

With the implemented methodology, it was possible to verify the presence of an oxidative stress in the *Foc* mycelium incubated with the culture filtrate (CF) of *B. amyloliquefaciens* CCIBP-A5. This type of damage could be verified by the significant increase ($p<0.05$) of the malondialdehyde (MDA) concentrations in the mycelial suspensions treated with the bacterial CF (Figure 3).

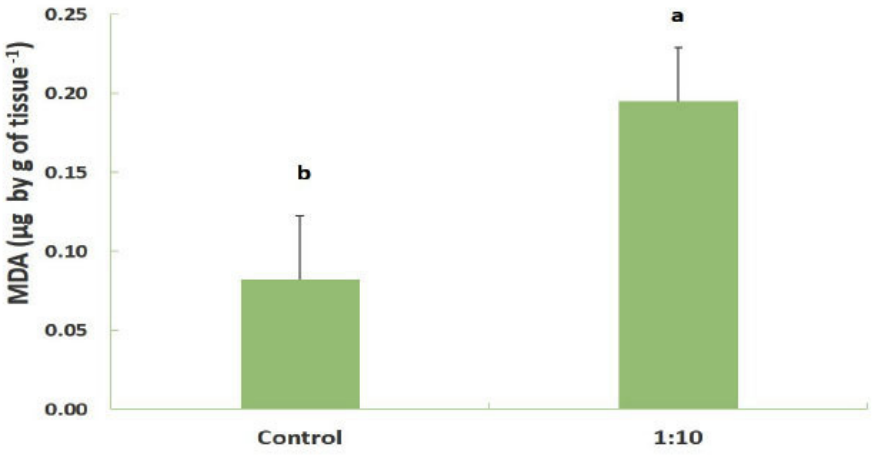

FIGURE 3
Production of malondialdehyde (MDA) as a result of the lipoperoxidation of the mycelium of *F. oxysporum* race 1 in presence of culture filtrate (CF) of *B. amyloliquefaciens* CCIBP-A5 at 48 h of incubation. Different letters in the columns indicate significant differences ($p<0.05$) according to Kruskal-Wallis H test (n=8) and the vertical bars indicate standard deviation.

Figura 3. Producción de molondialdehido (MDA) como resultado de la lipoperoxidación del micelio de *F. oxysporum* raza 1 en presencia del filtrado de cultivo (CF) de *B. amyloliquefaciens* CCIBP-A5 a las 48 h de incubación. Letras diferentes en las columnas indican diferencias significativas ($p<0,05$) según Kruskal-Wallis (n=8) y las barras verticales indican la desviación estándar.

Antifungal potential of the strain in plants under controlled conditions

As a result of the evaluation of the experiment 22 days after the inoculation (dpi), typical symptoms of Fusarium wilt were found in the plants used for the study. These symptoms varied according to the treatments as shown in Figure 4.

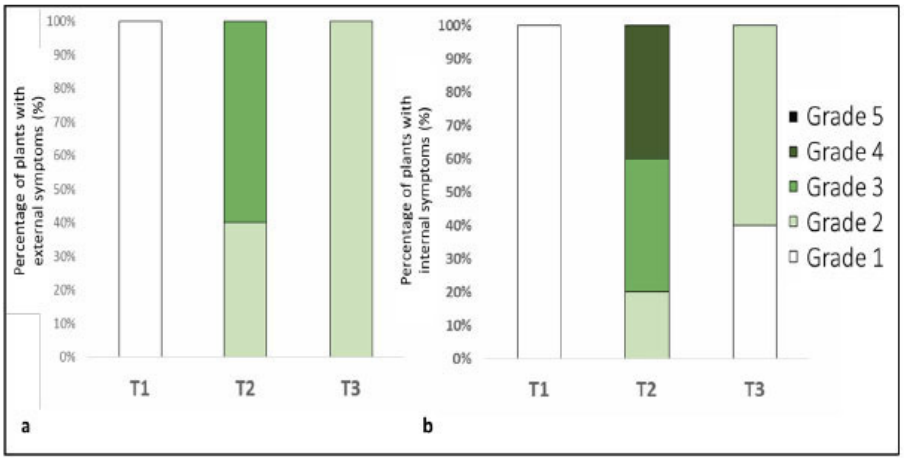

FIGURE 4
Evaluation of the symptoms of Fusarium wilt in Gros Michel plants according to the scale proposed by Dita et al. (2014) at 22 dpi under controlled conditions. Laboratory of Applied Microbiology, Instituto de Biotecnologia de las Plantas, Cuba, 2018. (a) External symptoms (b) Internal symptoms. T1: Control; T2: Plants inoculated with *F. oxysporum* race 1; T3: Plants inoculated with *F. oxysporum* in the presence of *B. amyloliquefaciens* CCIBP-A5.

Figura 4. Evaluación de los síntomas de la Marchitez por Fusarium en plantas de Gros Michel de acuerdo a la escala propuesta por Dita et al. (2014) a los 22 dpi bajo condiciones controladas. Laboratorio de Microbiología Aplicada, Instituto de Biotecnología de las Plantas, Cuba, 2018. (a) Síntomas externos (b) Síntomas Internos. T1: Control; T2: Plantas inoculadas con *F. oxysporum* raza 1; T3: Plantas inoculadas con *F. oxysporum* en presencia de *B. amyloliquefaciens* CCIBP-A5.

The results indicated that strain CCIBP-A5 had an effect on the development of Fusarium wilt disease in Gros Michel plants under controlled conditions. At 22 dpi it was observed that the inoculated plants where the bacterium was not applied (T2) 60 % of them reached the grade 3 and 40 % grade 2, while in 100 % of the plants in the presence of the bacterial strain (T3) showed external symptoms only in grade 2.

When the internal symptoms were analyzed, greater differences were observed between the treatments in relation to the degrees of the disease in the same evaluation period (Figure 4). For example, 100 % of the plants inoculated with *Foc* (T2) showed typical internal symptoms of the disease according to the scale used, which varied from grade 2 to grade 4 with grade 3 and 4 predominating by 40 %. In contrast, in the case of plants in the presence of CCIBP-A5, only 60 % of them showed typical symptoms of the disease and corresponding only to grade 2.

Discussion

The antifungal assay results of *B. amyloliquefaciens* CCIBP-A5 match those of a study conducted by Abdallah et al. (2017), which demonstrated (using a different methodology) that culture filtrate (CF) of a strain of *B. amyloliquefaciens* was able to significantly reduce mycelial growth in *F. oxysporum* f. sp. *lycopersici*. Other studies also demonstrate the inhibitory effects of the diffusible metabolites of *Bacillus* spp. present in CF against Fusarium species (Nourozian et al., 2006).

The changes observed in the morphology of the *Foc* mycelium have been described by several authors in other phytopathogenic agents. For example, a study by Liao et al. (2016) noted that malformations occurred in hyphae of Pyricularia oryzae Cavara such as swelling due to the presence of lipopeptides type fengicinas. These results also coincide with those obtained by Tang et al. (2014). On the other hand, Li et al. (2005), showed that a lipopeptide antibiotic produced by a strain of *B. subtilis*, induced the formation of chlamydospores in *Trichoderma harzianum* Rifai and *Glioladium roseum* at low concentrations

Chlamydospores are survival structures that originate in response to unfavorable environmental conditions. The production of these structures induced by the presence of CF of the strain CCIBP-A5, suggests a stressful effect on the mycelium of *Foc*, however, these results may not be the most favorable for the application of CF in ex vitro conditions. Therefore, *Bacillus* and its lipopeptides can have an important impact on the microbe interaction in soils, not always with desired results. In general, other studies are required to determine the viability of these chlamydospores in the presence of CF of the bacterial strain.The changes observed in the morphology of the *Foc* hyphae in the presence of CF of strain CCIBP-A5, could be related to the action of a metabolite of protein nature produced by the strain, present in the filtering.

The absorbance values obtained from the conidial suspension in the presence of CF of CCIBP-A5 suggested that the compounds present in CF have a negative effect on *Foc* conidia, even at very low concentrations. Both the 1:10 and 1: 100 dilutions reduced the absorbance by more than 36 % compared to the control. Although this methodology constitutes an indirect measure to evaluate the germination of the conidia. Those effects were similar to observed in conidia of other Fusarium species in presence of CF of *B. amyloliquefaciens* strains (Abdallah et al., 2017; Lee et al., 2017). In the presence of CF, deformations were observed in the germ tubes of the conidia related to the winding and sometimes the lysis of these structures. According to Li et al. (2012) the presence of lipopeptides at concentrations close to the minimum inhibitory, caused lysis of conidia and mycelium in two species of Fusarium. Similar results were described by Liao et al. (2016) who demonstrated that lipopeptide compounds such as fengicina can cause deformations in the germ tube of the conidia of fungal agents.

The effect of metabolites on conidial germination and mycelial growth was observed, even using very low concentrations of CF. However, the effectiveness of the metabolites presents in the CF, capable of inhibiting the growth and development of *Foc* at very low concentrations, may have unexpected results on soils (Li et al., 2012).

The type of damage observed could be confirmed by the significant increase in the concentrations of MDA in the MS treated with CF. Lipids represent a group of molecules very susceptible to damage caused by reactive oxygen species, mainly those of unsaturated type which are oxidized very easily (Yin et al., 2011). Different types of environmental stress can lead to oxidative stress, which in turn stimulates lipid peroxidation processes. The fungal membranes are rich in polyunsaturated fatty acids, so this process can cause direct damage to the structures of the cell membrane and/or indirectly to other cellular components. One of the final products of the peroxidation of unsaturated fatty acids is MDA, which has been used as one of the markers of this type of stress in animal and vegetable tissues (Ayala et al., 2014).

Taking into account these criteria and the results obtained with MDA values, it could be inferred that the antifungal effect of the metabolites present in the CCIBP-A5 CF may be partially related to the oxidative damage, mainly in the lipid compounds of plasma membrane of *Foc*. It has been shown that some changes in the plasma membrane represent the first step of a cascade of events that promote an internal osmotic imbalance and a disorganization in the cytoplasm. Also, it is characteristic that these processes induce an abundant vacuolization, the increase of the cytoplasm aggregation and the loss of organelles (Wang et al., 2002). In this way, the morphological deformations observed on the *Fusarium* mycelium in the presence of CF could be also related in part to oxidative damage. Furthermore, with the in vivo experiment, it was possible to verify that the strain had the effect of reducing the development of the disease in artificially inoculated plants. This was evidenced by the decrease in the number of plants with symptoms of the disease (external and internal), as well as in the degrees of the disease in the same evaluation period. These results could be related to the antifungal effect observed *in vitro*, of the metabolites produced by this strain. In this sense, several studies have reported the potentialities of strains of the *Bacillus* genus to promote plant growth (Rojas-Solís et al., 2013). These effects can be divided into direct and indirect mechanisms. The first are those where the bacteria can positively influence the growth of the plant through the synthesis and excretion of phytostimulatory substances. Likewise, indirect methods are those where the bacteria synthesize antibiotics or other compounds that have an inhibitory effect on phytopathogenic organisms and even that strengthen plant immunity. Specifically, *B. amyloliquefaciens* has been indicated as an antagonistic species of plant pathogens through mechanisms such as: competition for essential nutrients (Wu et al., 2016), production of antibiotics (Srivastava et al., 2016) and induction of systemic resistance in plant (Ng et al., 2016).

Conclusion

The results of this research indicated that the metabolites present in the culture filtrate (CF) of *B. amyloliquefaciens* CCIBP-A5 significantly influenced the growth and morphology of the mycelium and the conidia. They also caused oxidative damage in the lipid molecules of *F. oxysporum* f. sp. *cubense* race 1 which could be related to the morphological damage observed in the mycelium. Taking into account previous studies, it is suggested that the observed antifungal activity could be partly associated with the action of lytic enzymes and /or lipopeptide compounds present in the CF.

B. amyloliquefaciens CCIBP-A5 strain, isolated from *Musa* sp, has an *in vitro* antifungal effect against *F. oxysporum* as well on the *Musa* spp. - *F. oxysporum* interaction. These aspects are key when selecting a bacterial candidate as a biological control agent. That's why, these results constitute a starting point for the use of this strain for the development of a bioproduct for the management of Fusarium wilt.

Compliance with ethical standards

The research does not involve any human participants or animals. The materials in the article have not been published in another journal. All authors have been actively involved in substantive work leading to the manuscript and will hold themselves jointly and individually responsible for its content.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Notes

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