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Antifungal activity of endophytic microorganisms isolated from *Acmella ciliata* (Asteraceae)

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ABSTRACT. The study of microorganisms that inhabit the interior of plants (endophytes) has acquired great importance because of their potential to produce bioactive metabolites. *Acmella ciliata* (known in Brazil as jambu) is a native herb of South America, used in regional gastronomy and folk medicine in Amazonas - Brazil, has antibacterial properties, and may be a useful host for bioactive endophytes. We isolated endophytic microorganisms from *A. ciliata* and evaluated their ability to inhibit pathogenic fungi. We isolated 56 fungi and 39 endophytic bacteria, most of them from the leaves. The endophyte isolates were then tested in antagonism assays against the phytopathogens *Fusarium decemcellulare* and *Colletotrichum gloeosporioides*. The best antagonism index values against *C. gloeosporioides* were obtained with the endophytic fungus UEA-253 (40%), and the endophytic bacterium UEA-135 (46.3%). The highest antagonism index values were obtained against *F. decemcellulare* with the fungus UEA-234 (47.2%), and the bacterium UEA-135 (44.8%). The endophytic fungi with inhibitory bioactivity belong to the genera *Curvularia*, *Colletotrichum*, *Plectosphaerella* and *Sordariomycetes*, while the endophytic bacteria belong to the genera *Bacillus*, *Pseudomonas* and *Enterobacter.* We conclude that the endophytic fungi and bacteria isolated from *A. ciliata* have potential for use in the biocontrol of *F. decemcellulare* and *C. gloeosporioides*.

Key words: Fungus; Phytopathogen; Biocontrol; *Fusarium decemcellulare*; *Colletotrichum gloeosporioides*

INTRODUCTION

Endophytic microorganisms have been considered a promising source for the development of biological control agents against phytopathogens because they exhibit these beneficial functions in host plants and are ubiquitous, being found in almost every tissue type studied. Even so, endophytic microorganisms are still a poorly investigated group, and their complex ecological functions have not been extensively exploited, though endophytic fungi apparently can help the host survive (Zheng et al., 2017). Endophytic microorganisms colonize the healthy tissues of plants at some point in their life cycle without causing any apparent damage (Petrini, 1991). Endophytes that inhabit plants with medicinal or antimicrobial properties have a high chance of producing bioactive metabolites (Azevedo et al., 2000). In this context, endophytic microorganisms have high potential for use in agriculture and industry, as they produce bioactive metabolites with anti-phytopathogenic activity that can inhibit pathogens such as *Cladosporium cladosporioides and C. sphaerospermum* (Zanardi et al., 2012), *Fusarium oxysporum* f. sp. *Lycopersici* (Sousa et al., 2013), *Sclerotinia sclerotiorum* and *Fusarium oxysporium* (Chowdhary and Kaushik, 2015), *Rhizoctonia solani*, *Sclerotinia sclerotiorum* (Cao et al., 2016), *Phythium myriotylum* (Sabu et al., 2017), *Alternaria solani* and *Fusarium oxysporium* (Yang et al., 2018). Campanile et al. (2007) suggests that interactions between plants and microorganisms are complex.

Acmella ciliata (Asteraceae), known in Brazil as “jambu", “agrião-do-pará”, and “agrião-do-brasil” is an important herb native to South America. Jambu is consumed only in northern Brazil, and its cultivation is well established; the city of Autazes is the main place of cultivation of this plant in Amazon state. The leaves and stem are commonly used in the gastronomy of the Amazon region because of their acrid taste. Is also known by the natives as a folk medicine to treat anemia, scurvy, tooth and throat ache, gum inflammation, and as an anesthetic and analgesic (Cardoso and Garcia, 1997; Favoreto and Gilbert, 2010; Rincón et al., 2012). Most Asteraceae species have antimicrobial activity (Rani and Murty, 2006). Several studies have found that *A. ciliata* and other species belonging to this genus have antibacterial (Rincón et al., 2012; Lalthanpuii et al., 2017), insecticidal and antimicrobial (Prachayasittikul et al., 2009; Alcantara et al., 2015; Anholeto et al., 2017; Marchesini et al., 2018; Benelli et al., 2019) and anti-phytopathogenic activities (Rani and Murty, 2006).

The use of microorganisms to biologically control phytopathogens is well known and of a great interest because this method minimizes environmental damage compared to the agrochemicals that are commonly used (Ulloa-Ogaz et al., 2015). *Colletotrichum* and *Fusarium* are the most troublesome genera of fungal plant pathogens, causing severe diseases in numerous economically important crops, and it is necessary to find alternatives to deal with it (Alabouvette et al., 2009; Ajilogba and Babalola 2013; Kejela et al., 2016).

To the best of our knowledge, there have been no studies on the endophytic microorganisms present in *A. ciliata*, and whether these endophytes have antimicrobial potential. In this context, *A. ciliata* was evaluated for potential antimicrobial activity of its endophytic microorganisms.

MATERIAL AND METHODS

Plant material

We randomly selected five healthy jambu plants, from farms in the city of Autazes, Amazonas (3°57'97" S, 59°13'06" E. The botanical identification was performed by Ramos J.F. of Instituto Nacional da Pesquisas Amazônia (INPA), and a dried specimen was deposited in the INPA herbarium under code OJEDA, CPO 1, voucher INPA N°. 274113.

Isolation of endophytes

The plant material was processed within 24 h. From each individual, 30 leaves and 30 stems were selected. The surface of the plant material was washed with tap water to remove the epiphytic microorganisms and soil. Disinfection was carried out by immersion in 70% ethanol for 1 min, sodium hypochlorite 2-2.5% for 4 min and 70% ethanol for 30 s (Pimentel et al., 2006). Thereafter, the plant material was submerged in sterile distilled water three times for 1 min each, and a 100 µL aliquot was seeded on potato dextrose agar (PDA) and tryptic soy agar medium (TSA) for control of asepsis. Six fragments of each leaf and each stem (5 x 5 mm) were cut and placed in Petri dishes containing PDA plus 100 $\mu g.mL^{-1}$of chloramphenicol for fungal isolation. For isolation of bacteria, the fragments were inoculated on solid tryptic soy broth (TSB) containing agar supplemented with cyclohexemide at a concentration of 100 $\mu g.mL^{-1}$, and the plates were incubated at 28°C.

Identification of endophytes

Identification of the fungus is based on the micromorphological features of the isolates, which were selected according to the different macro morphological characteristics shown in the potato dextrose agar culture media (PDA), (Kern and Blevins, 1999; Sbravatti et al., 2013). The selected fungi were grown in PDA and the DNA was extracted for amplification of internal transcribed spacer (ITS) regions ITS1, 5'-CGT AAC AAG GTT TCC GTA GG-3' and ITS4, 5'-TCC TCC GCT TAT TGA TAT GC-3',according to Harju et al. (2004) and Cassa-Barbosa et al. (2015). The amplification products were sequenced at the Biotechnology Laboratory of Universidade Federal do Amazonas (UFAM). DNA sequences have been deposited in the database of GenBank, and a phylogenetic tree was constructed using a hierarchical method for multiple alignments (Neighbor Joining), based on the sequences and strain patterns obtained. Phylogeny was tested with 500 bootstrap replications using the Molecular Evolutionary Genetics Analysis (MEGA-5) software (Tamura et al., 2011) to identify relationships among strains. The results of the amplification of the ITS regions of the isolates were used to construct the phylogenetic tree of these fungi, which showed high similarity with fungi deposited in the National Center for Biotechnology Information (NCBI).

From the isolated endophytic bacteria, we selected those which showed the ability to inhibit the growth of the pathogens in a preliminary assay (data not shown). The isolated and selected endophytic bacteria were seeded in tubes containing TSB and incubated at 28°C under constant stirring for 24 h. The DNA was extracted using CTAB buffer (1M Tris-HCl pH 8.0, 0.5 M EDTA, 1.4 M NaCl, 2% CTAB (cetyltrimethylammonium bromide), 1% PVP (polyvinylpyrrolidone)). The 16S region of each sample was amplified with primers 63F (5'-CAG GCC TAA CAC ATG CAA GTC-3') and 1389R (5'-ACG GGC GGT GTG TAC AAG-3'), using polymerase chain reaction according to Procópio et al. (2012).

The amplification products were also sequenced at the Biotechnology Laboratory of UFAM. DNA sequences were deposited in the database of GenBank, and the aligned sequences were compared with those of GenBank by the BLAST program to find homologies with sequences from related organisms. For the construction of the phylogenetic tree, the MEGA 5 software was used (Tamura et al., 2011).

Antagonism tests for endophytic fungi

To evaluate the antagonistic potential of the endophytic fungi, a paired culture test was carried out in Petri dishes (Φ 9.0 cm) containing PDA medium, where discs (Φ 0.5 cm) were cultured with three days of growth of the selected endophytic fungi and of the phytopathogens test *Colletotrichum gloeosporioides* and *Fusarium decemcellulare* were kindly provided by Professor Rogério Hanada, INPA. The distance between the phytopathogen and the endophytic fungus was 4 cm. As a negative control, a PDA disc and a disk containing the pathogen were placed at the same distance and incubated at 28°C, during the time necessary for the control plate containing only the phytopathogen to reach the opposite end of the Petri dish. Each combination of endophyte and phytopathogen was tested in triplicate. Colony growth was measured at intervals of 24 h. The Antagonism Index (AI) was calculated according to the following formula: AI = (RM−rm)/RM×100, where rm = radius of the colony towards the antagonist and RM = mean of three radiuses of the colony in various directions, as described by Campanile et al. (2007).

Antagonism tests for endophytic bacteria

In order to evaluate the antagonistic capacity of endophytic bacteria, a qualitative analysis was first carried out, where agar discs (Φ 0.5 cm) were inoculated in the center of a Petri dish (Φ 9.0 cm) containing the test phytopathogens (*C. gloeosporioides* and *F. decemcellulare*). After 72 h, the isolated endophytic bacteria were inoculated in four quadrants. Finally, the dishes were incubated at 28°C for seven days. Bacteria that exhibited antagonism against phytopathogens were tested in individual assays, which consisted of inoculating a drop of 10 µL of the bacterial solution (10^8 bacterial cells mL^{-1} or 0.5 on the McFarland scale) at the edge of the Petri dish and placing an agar disk (Φ 0.5 cm) containing the phytopathogen in the center of the dish. The dishes were incubated for 5 to 7 days at 28°C in triplicate. The AI was calculated by the formula described by Silva et al. (2016).

RESULTS

Isolation of endophytic fungi

A total of 56 endophytic fungi were isolated from the 360 leaf and stem fragments, 40 obtained from the leaves and only 16 from the stems. From the total of isolates, 28 different fungi were selected, taking into account the macro and micro-morphological criteria. Through the micro-culture test it was possible to identify the genera *Curvularia* and *Colletotrichum.*

For the DNA analyses, after several tries, it was only possible to extract the DNA of 14 isolates. In the molecular identification of the 14 isolates, four genera were identified, *Colletotrichum* (nine isolates), *Curvularia* (three isolates), *Plectosphaerella* and *Sordariomycetes*, the latter two with only one representative each (Figure 1 and Table 1). In the case of the fungus UEA-246, despite several attempts, extraction of the DNA was not successful.

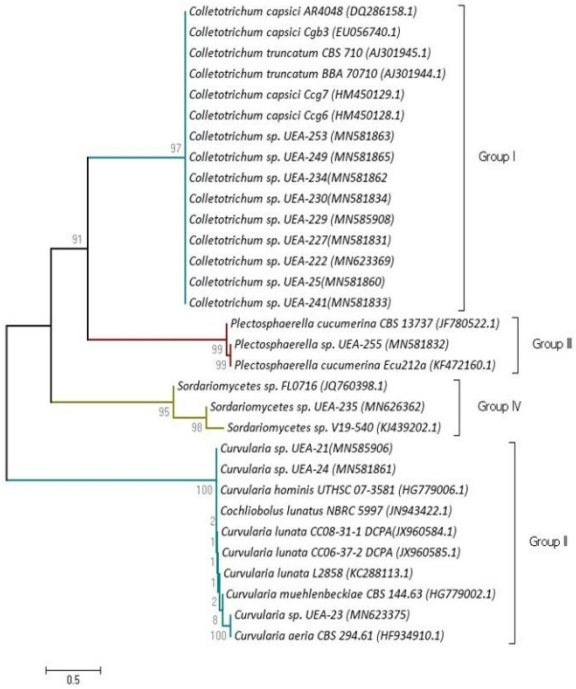

Figure 1. Phylogenetic tree based on sequences from the ITS region of endophytic fungal isolates from *Acmella ciliata*.

Table 1. Code of isolated fungi, identification, deposit code in GenBank, part of the plant where the fungus was isolated, and fungus strains with high similarity in GenBank.

CODE	IDENTITY	GENBANK CODE	SITE OF ISOLATION	HIGEST IDENTITY GENBANK
UEA-241	*Colletotrichum* sp.	MN581833	leaf	99% *Colletotrichum capsici* GK17A MK713419
UEA-24	*Curvularia* sp.	MN581861	leaf	100% *Curvularia hominis* UTHSC 07-3184 (HG779005)
UEA-229	*Colletotrichum* sp.	MN585908	leaf	100% *Colletotrichum capsici* GK17A (MK713419)
UEA-222	*Colletotrichum* sp.	MN623369	leaf	100% *Colletotrichum* sp. 70a (KT825856)
UEA-235	*Sordariomycetes* sp.	MN626362	leaf	95% *Sordariomycetes* sp. V19-540 (KJ439202)
UEA-21	*Curvularia* sp.	MN585906	leaf	98% *Curvularia* sp. OLS5 (KU898069.1)
UEA-227	*Colletotrichum* sp.	MN581831	leaf	100%*Colletotrichum truncatum* AGSV17 (MN298753)
UEA-230	*Colletotrichum* sp.	MN581834	leaf	100% *Colletotrichum truncatum* PRI180023 (MN148631)
UEA-25	*Colletotrichum* sp.	MN581860	leaf	100% *Colletotrichum truncatum* CCC38 (KX648386)
UEA-23	*Curvularia* sp.	MN623375	leaf	97% *Curvularia lunata* UFMGCB4427 (KJ404197)
UEA-249	*Colletotrichum* sp.	MN581865	leaf	100% *Colletotrichum truncatum* HB09 (KX364059)
UEA-234	*Colletotrichum sp.*	MN581862	leaf	99% *Colletotrichum truncatum* CCC38 (KX648386)
UEA-253	*Colletotrichum* sp.	MN581863	leaf	100%*Colletotrichum truncatum* AGSV17 (MN298753)
UEA-255	*Plectosphaerella* sp.	MN581832	stem	100% *Plectosphaerella cucumerina* Ecu212a (KF472160)

Isolation of endophytic bacteria

Initially it was possible to isolate 81 endophytic bacteria from 360 leaf and stem fragments obtained from the plants, of which 50 were from stems and 31 from the leaves. For antagonism tests, it was possible to cultivate only 39 bacteria. Of the 39 cultured endophytic bacteria, 16 were selected for identification. Based on phylogenetic analysis of 16S rDNA, the selected bacteria were grouped into three genera: *Bacillus*, *Pseudomonas* and *Enterobacter*, by comparison with standard strains (Figure 2 and Table 2). Isolate UEA-120 from *Bacillus* presented the largest difference within the genus. The isolates of *Pseudomonas* (UEA-135 and UEA-139) had 100% similarity to *P. aeruginosa*. *Enterobacter* (UEA-114 and UEA-134) also presented 100% similarity. The three genera showed high reliability of the data, with a bootstrap value of 99.

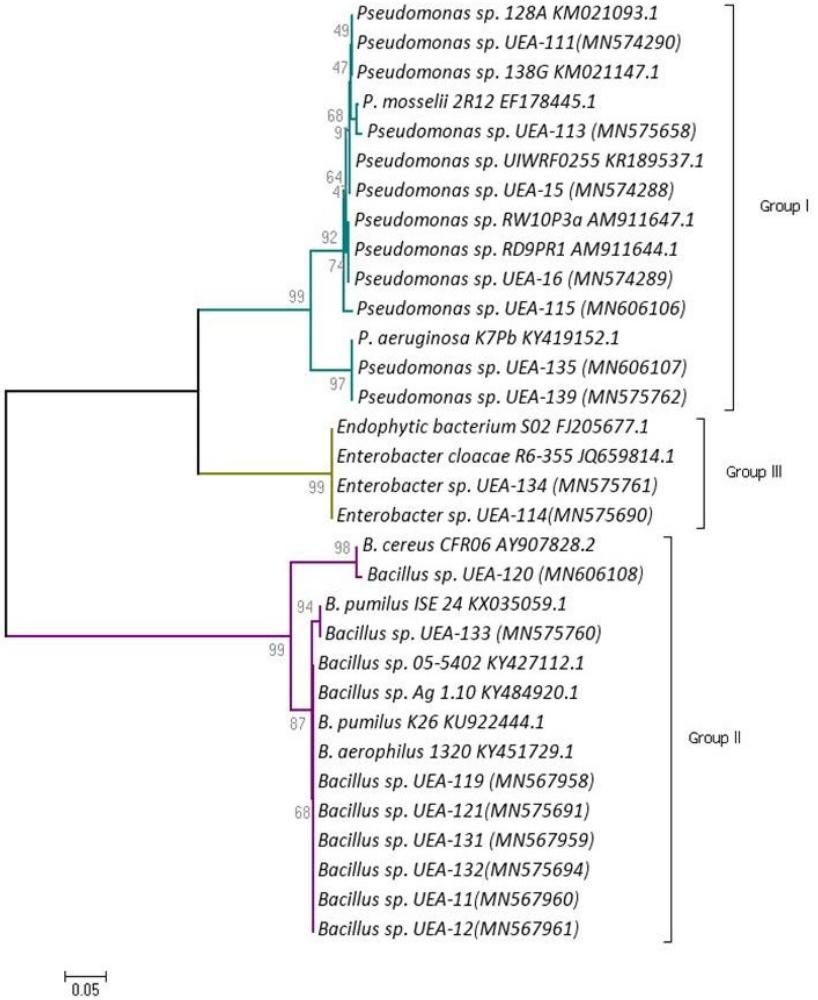

Figure 2. Phylogenetic tree based on the 16S rDNA sequences of jambu endophytic bacterial isolates considering Neighbor Joining.

Table 2. Code of isolated bacteria, identification, deposit code on GenBank, part of the plant where the bacteria was isolated, and bacterial strains with high similarity on GenBank.

CODE	IDENTITY	GENBANK CODE	SITE OF ISOLATION	HIGEST IDENTITY GENBANK
UEA-15	*Pseudomonas* sp.	MN574288	stem	99% *Pseudomonas* sp. P3 (MN400354)
UEA-16	*Pseudomonas* sp.	MN574289	stem	99% *Pseudomonas* sp. AA139 (MN540111)
UEA-12	*Bacillus* sp.	MN567961	stem	99% *Bacillus cereus* McL6 (KY078799)
UEA-11	*Bacillus* sp.	MN567960	stem	100% *B. altitudinis* HRG-1(MN590432)
UEA-133	*Bacillus* sp.	MN575760	stem	99% *Bacillus safensis* N32 (MN555373)
UEA-134	*Enterobacter* sp.	MN575761	stem	99% *Enterobacte*r sp. GJ1-11(EU139848)
UEA-111	*Pseudomonas* sp.	MN574290	stem	99% *Pseudomonas* sp. JL-1 (MN192065)
UEA-139	*Pseudomonas* sp.	MN575762	stem	99% *P. aeruginosa* K7Pb (KY419152)
UEA-135	*Pseudomonas* sp.	MN606107	stem	99% *P. aeruginosa* MV18 (KR061897)
UEA-121	*Bacillus* sp.	MN575691	leaf	99% *Bacillus aerius* RPW17(MN582994)
UEA-115	*Pseudomonas* sp.	MN60610	leaf	97% *P. putida* AAU PR2 (KJ161326)
UEA-114	*Enterobacter* sp.	MN575690	leaf	99% *E. cloacae* NIBSM (KY930712)
UEA-131	*Bacillus* sp.	MN567959	leaf	99% *Bacillus aerius* RPW17 (MN582994)
UEA-132	*Bacillus* sp.	MN575694	leaf	99% *B. stratosphericus* HR61(KM261764)
UEA-113	*Pseudomonas* sp.	MN575658	leaf	99% *Pseudomonas* sp. UPMCB-A0024 (KY784626)
UEA-120	*Bacillus* sp.	MN606108	leaf	99% *Bacillus cereus* CFR06 (AY907828)
UEA-119	*Bacillus* sp.	MN567958	leaf	99% *B. altitudinis* HRG-1(MN590432)

Antagonism tests for endophytic fungi

Of the 28 endophytic fungi tested in the pairing of colonies with *C. gloeosporioides* and *F. decemcellulare*, 21 (75%) presented antagonism (Figure 3). In the pairings against *C. gloeosporioides*, the endophytic fungus UEA-253 presented the highest AI, 39.9%. Furthermore, the endophytic fungi UEA-234, UEA-246 and UEA-249 presented AIs of 38.7, 38.5 and 38.5% respectively.

The differences between the values of AI of these fungi were not significant (Figure 4). In the evaluation against *F. decemcellulare*, the results of AI also were classified into three ranges (10-30%, 30-40% and 40% or more). In the highest range, the endophytic fungus UEA-234 obtained AI of 47.2%, the highest value in this test. Also, the endophytic fungi UEA-253obtain the second highest value of AI with less variation between the repetitions (46.5%). The differences between the values shown by UEA-253 and UEA-234 were not significant (Figure 4). The endophytic fungi with antagonistic activity obtained a higher index against *F. decemcellulare* in comparison with the values obtained in the pairings against *C. gloeosporioides* (Figure 4).

Four days after the start of the antagonism test between *F. decemcellulare* and the endophytes UEA-21, UEA-23, UEA-24, UEA-25, UEA-27, UEA-28, UEA-212, UEA-229 and UEA -253, the phytopathogen changed from pink to yellow color (Figure 5).

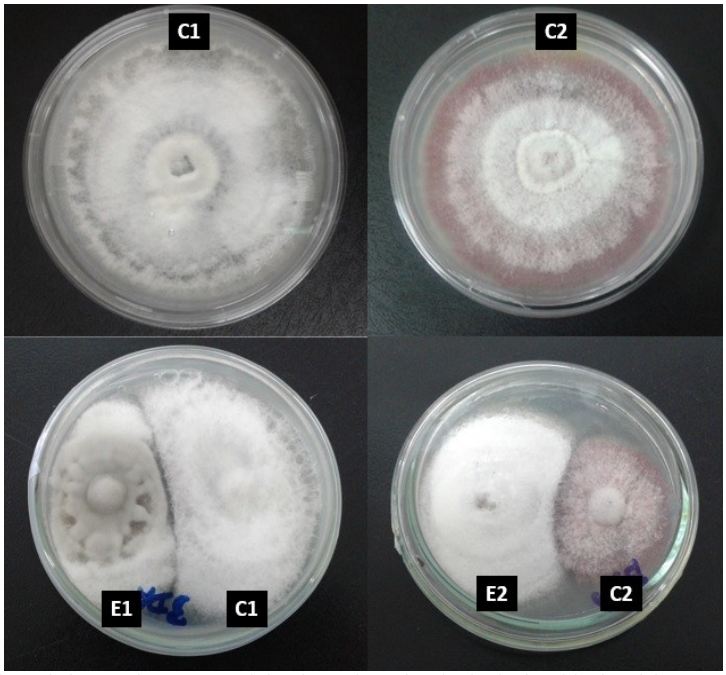

Figure 3. Inhibition of phytopathogen growth by the action of endophytic fungi isolated from *Acmella ciliate*, C1: *Colletotrichum gloeosporioides*, C2: *Fusarium decemcellulare*, E1: endophytic fungus UEA-234, E2: endophytic fungus UEA-227.

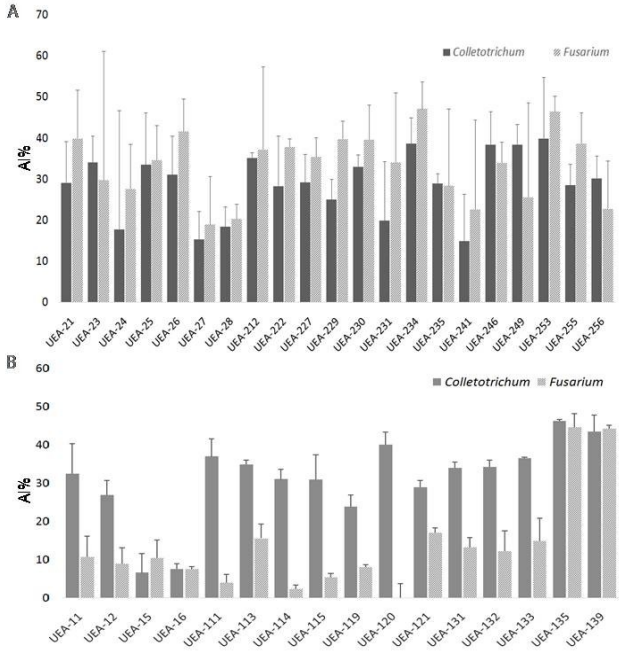

Figure 4. Antagonism index (AI) of endophytic fungal (A) and bacteria (B) isolates from *Acmella ciliata* in the pairing with *Colletotrichum gloeosporioides* and *Fusarium decemcellulare*.

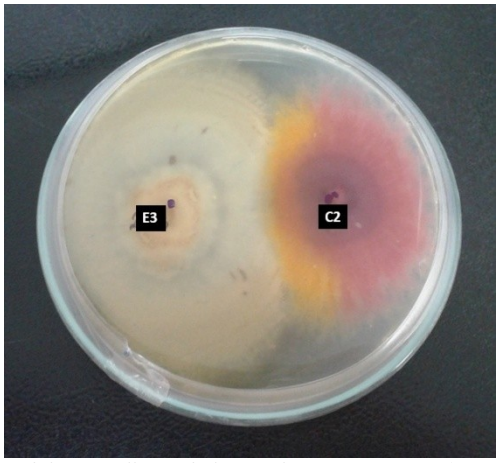

Figure 5. Change in the color of the mycelium of phytopathogen *Fusarium decemcellulare*. C2: Phytopathogen *Fusarium decemcellulare*, E3: endophyte UEA-253.

Antagonism tests for endophytic bacteria

Of the 39 cultured endophytic bacteria, only 16 presented antagonistic activity against phytopathogenic fungi. From the preliminary assay, 16 isolates presented positive results for inhibition of the growth of the phytopathogen *C. gloeosporioides* and 15 for *F. decemcellulare*. In individual antagonism assays with *C. gloeosporioides*, all 16 bacteria inhibited the growth of the phytopathogen. The bacteria UEA-120, UEA-135 and UEA-139 obtained the highest AI values, with values of 40.1, 46.3 and 43.5%, respectively. There were significant differences in the comparisons between the AI of strains UEA-120 and UEA-135, and UEA-120 and UEA-139, but there were no significant differences between the strains UEA-135 and UEA-139 (Figure 6).

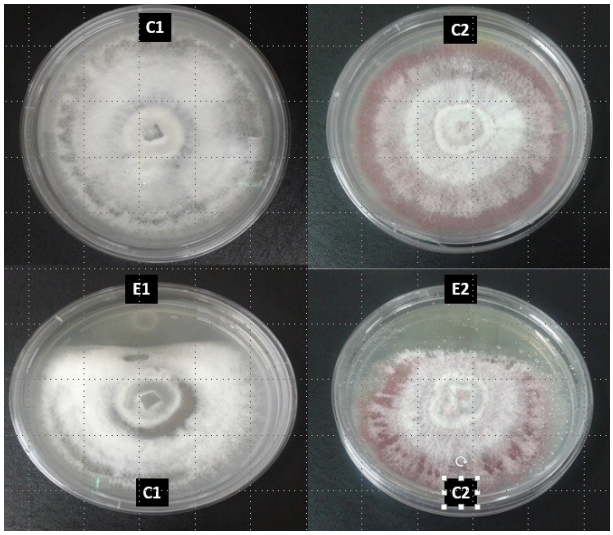

Figure 6. Inhibition of phytopathogen growth by the action of endophytic bacterial isolates from *Acmella ciliate*, C1: *Colletotrichum gloeosporioides*, C2: *Fusarium decemcellulare*, E1: endophytic bacterium UEA-135, E2: endophytic bacterium UEA-139.

In the tests carried out with *F. decemcellulare*, the bacteria UEA-135 and UEA-139 obtained the highest levels of AI (44.7 and 44.3% respectively). There were no significant differences between the AIs of these strains. In this case, the UEA-120 bacterium showed no growth inhibition against *F. decemcellulare*. The AI found with *C. gloeosporioides* generally was superior to the AI with *F. decemcellulare* (Figure 4). The inhibition caused by the UEA-135 and UEA-139 bacteria were very similar for the two phytopathogenic fungi, where in the case of bacteria UEA-15 and UEA-139, the AI against *F. decemcellulare* was higher than the AI presented against *C. gloeosporioides*. In all other cases, inhibition of growth was higher against *C. gloeosporioides*. None of the endophytic bacteria with positive results for growth inhibition exceeded 50% antagonism.

DISCUSSION

The diversity and biocontrol potential of cultivable endophytic fungi and bacteria harbored in *A. ciliata* plants are here described for the first time. Most of the fungi and bacteria were isolated from the leaves (2/3). The difference in the frequency of isolates from leaves versus stems may be due to the leaf stomata, which are natural entryways for microorganisms (Santos and Varavallo, 2011). A similar result was obtained by Souza et al. (2004), who isolated endophytic fungi and bacteria from Amazon plants (*Palicourea longiflora* and *Strychnos cogens*), with the highest frequency of microorganisms obtained from the leaves, compared to the number of microorganisms obtained from the stems and roots. John and Mathew (2017) also observed greater frequency of endophytic fungi in leaves of *Achyranthes aspera*, followed by stems and roots, the most frequent genus being *Colletotrichum*. The genera *Colletotrichum, Curvularia, Plectosphaerella and Sordariomycetes,* which have been found in leaves and stem of *A. ciliata*, are common in other plant species, such as *Theobroma cacao* (Hanada et al., 2010), *Sapindus saponaria* (García et al., 2012), *Musa acuminate* (Zakaria et al., 2016), *Terminalia laxiflora* (Tawfike et al., 2018), *Glycine max* (Fernandes et al., 2015), *Catharanthus roseus* (Ayob and Simarani, 2016), *Pogostemon cablin* (Wang et al., 2017a), *Hancornia speciosa* (Chagas et al., 2017). These fungi are of similar genera to those found in this work.

The three genera of bacteria (*Pseudomonas, Bacillus* and *Enterobacter*) recorded in our study have also been reported in other plant species (Goryluk-Salmonowicz et al., 2016), including medicinal plants such as *Trichilia elegans* (Rhoden et al., 2015). These bacteria have been shown to be important growth promoters in plants (Pereira et al., 2012), besides inhibiting phytopathogens (Nongkhlaw and Joshi, 2014; Zhao et al., 2017). The inhibitory effect on phytopathogen growth can be attributed to the possible production of chitinases or other enzymes with action against the fungal cell wall (Fuga et al., 2011), or due to different mechanisms such as synthesis of antimicrobial substances, competition for space and nutrients, secretion of lytic enzymes, pH alteration or synthesis of volatile compounds (Souza et al., 2015).

Endophytic bacteria belonging to these genera also have shown antimicrobial activity against phytopathogens (Li et al., 2012; Chen et al., 2014; Nascimento et al., 2015). The endophytes isolated from *Brassica campestris* showed the strongest antagonistic reactions against the pathogens *Bacillus cereus* HNR10, *Pseudomonas* sp. HNR13, and *B. subtilis* (TPR02, TPR03), and also had strong antagonistic activity against the fungi *Phythium ultimum, Phytophthora capsici, Fusarium oxysporum*, and *Rhizoctonia solani*

(Haque et al., 2016). Jasim et al. (2016) observed that the endophytic bacterium CaB5 (*Bacillus* sp.) isolated from *Capsicum annuum* showed inhibition against *Fusarium* sp. among other pathogens. In China, three endophytic species of *Bacillus thuringiensis* were isolated from wheat plants. Two of the three species of *Bacillus* were efficient for control of the phytopathogen *Urocystis tritici* Körn, which causes wheat flags mut (WFS), meaning it is an alternative for the biological control of this disease attacks wheat crops (Aili et al., 2014). Regarding *Pseudomonas*, Zhou et al. (2014) evaluated the activity of the endophytic bacterium *P. fluorescens* against the mycelial growth of the phytopathogenic fungus *Athelia rolfsii* (found in soil and responsible for rotting and wilt in various Chinese medicinal plant species). The results showed that *P. fluorescens* was able to inhibit the growth of *A. rolfsii* by contact and also by the production of volatile metabolites. Microscopic observations revealed the rupture and partial dissolution of the mycelium, preventing the growth of the fungus.

Bacillus subtilis isolated from inhibited mycelial growth of eight phytopathogenic species of *Fusarium*, causing abnormalities in the hyphae, such as swelling of filaments and tips, causing vacuolization (Chan et al., 2003). *Bacillus* species (BT42) isolated from *Coffea arabica* L. rhizosphere were capable of counteracting the pathogenic effects of *Colletotrichum gloeosporioides* and *Fusarium oxysporium* by synergistic effects of secondary metabolites, lytic enzymes, and siderophores (Kejela et al., 2016).

Among the fungi evaluated for biological control, only the isolates UEA-253, UEA-249, UEA-246 and UEA-234 presented promising results against the tested phytopathogens. Of these isolates, UEA-253, UEA-249 and UEA-234 belong to *Colletotrichum*, also presenting high similarity with *C. capsici* and *C. truncatum*, with *C. capsici* and *C. truncatum* being considered phytopathogens (Calzadaet al., 2011; Rogério et al., 2016). It should be pointed out that phytopathogenic fungi can be found as endophytes (Wang et al., 2017b).

In previous studies of antagonism against the phytopathogen *C. gloeosporioides*, one of the endophytes, isolated from the guarana seed, obtained an AI of 52.41% (Silva, 2016), the maximum obtained among all endophytes isolated in that study. In our study, the highest rates of antagonism against *C. gloeosporioides* and *F. decemcellulare* (39.97 and 47.21%) were lower than those found in Silva (2016). In a study with endophytic bacteria isolated from *Echinodorus scaber*, the inhibition of phytopathogens, among them *F. solani* and *C. gloeosporioides*, was also observed, as well as the alteration in the color of the mycelium, could be probably by the contact between the microorganisms, vacuolization and cellular rupture (Souza et al., 2015). According to Silva (2016), the endophytic bacteria isolated from the guarana seed had growth inhibition percentages against *C. gloeosporioides* close to 32%.

CONCLUSIONS

The results obtained from the antagonism tests showed that the endophytic fungi UEA-253, UEA-234 and bacteria UEA-135, UEA-135 isolated from *A. ciliata* have the potential for use in the biocontrol of the phytopathogens *Fusarium decemcellulare* and *Colletotrichum gloeosporioides*.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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