Acta Scientiarum
http://periodicos.uem.br/ojs
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v43i1.51737

Ornamental plant *Pachystachys lutea* as a source of promising endophytes for plant growth and phytoprotective activity

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ABSTRACT. Endophytes are growth-promoting agents capable of synthesizing phytohormones, uptaking nutrients, and controlling pathogens. There is a strong potential to exploit them in the agriculture field like biofertilizers and biocontrol agents. In this work, we aimed to evaluate endophytic fungi isolated from *Pachystachys lutea* for their potential to solubilize phosphate, synthesise indole acetic acid (IAA), antagonize phytopathogens, and promote plant growth under greenhouse conditions. The phosphate solubilization efficiency was assessed on Pikovskaya's agar medium. For analysis of IAA production, mycelia plugs of endophytes were cultured in Potato Dextrose Broth medium supplemented with L-tryptophan, with Salkowski Reagent, and the absorbance of the culture was measured. The antagonism evaluation of strain *Alternaria* sp. PL75 against phytopathogens was performed using the paired-culture technique. The promotion of plant growth provided by *Alternaria* sp. PL75 was evaluated in tomato plants. All strains evaluated were able to solubilize phosphate; however, the strain *Alternaria* sp. PL75 was the most effective (4.29). Two strains, *Nemania* sp. PL27 and *Alternaria* sp. PL75, produced 1.86 and 1.73 μg mL^{-1} of IAA, respectively. In the antagonism assay, the endophyte *Alternaria* sp. PL75 and its fungal extract showed the best results against the pathogen *Moniliophthora perniciosa*. The greenhouse experiment result showed the endophyte *Alternaria* sp. PL75 increased the plantlets emergency speed index and the percentage of germination from 60 to 81.63%. It was also observed a statistical significance in the shoot length of the treated plants with the endophyte suspension (55.38 cm) compared to the control (41.67 cm).

Keywords: phosphate solubilization; IAA; endophytic fungi; plant growth; antagonism.

Received on January 8, 2020.
Accepted on June 12, 2020.

Introduction

Endophytes are symptomless microorganisms that colonize plant tissues at the least part of their lifestyle cycle, living inter- or extracellularly, without producing external structures or damaging their hosts (Santos et al., 2018; Yan et al., 2019). Endophytes have been isolated from different healthy plant tissues (roots, stems, leaves, flowers, fruits, and seeds), and fungi are most frequently isolated (Strobel, 2018).

Endophytic microorganisms can be used either directly to boost agricultural productivity through enhancing plant growth, increasing seed production, modulating the environmental effects caused by biotic and abiotic stress, or triggering biological control for diseases (Rai et al., 2014; Nandhini et al., 2018; Strobel, 2018). Their growth-promoting agent abilities make them extremely useful in agriculture (Strobel, 2018).

The interaction between endophytes and plants is well-characterized as a symbiotic relationship. From the perspective of the plant, these microorganisms fix nitrogen, solubilise phosphate, synthesise phytohormones (such as indole acetic acid - IAA). On the other hand, plants provide shelter and nutrients to the endophytes (Ikram et al., 2018; Nandhini et al., 2018; Adhikari & Pandey, 2019). Endophytic fungi, which perform different symbiotic and ecological functions, may contribute to the adaptation of plants to their environments (Santoyo, Moreno-Hagelsieb, & Orozco-Mosqueda, 2016; Adhikari & Pandey, 2019).

In agriculture, microorganisms capable of producing IAA and solubilizing phosphorous, thereby increasing the development of their host, have proven themselves useful in the approaches to friendly agriculture (Murphy, Doohan, & Hodkinson, 2018; Nandhini et al., 2018). Besides, several of these endophytes can antagonize pathogens and control different diseases (Santoyo et al., 2016; Murphy et al., 2018; Strobel, 2018).

Despite the many challenges in the commercialization of microbial endophytic inoculants, researchers have

yielded promising results that might reduce the damage in the environment and economic loss. Murphy et al. (2018) successfully developed an endophytic fungus inoculant from barley which increases the biomass production of plants cultivated under lack of nutrients, drought, temperature, and pathogen stress, and also overcomes the development of pathogens transmitted by seeds. Furthermore, there are many fungal and bacterial biological control agents available commercially (Silva, Brooks, Lumyong, & Hyde, 2019).

Thus far, the endophytic communities of around 1-2% of plants worldwide have been studied (Strobel, 2018). The ornamental plants have been less studied, and the previous investigations were mainly performed in Orchidaceae. Ribeiro et al. (2018) described the isolation of endophytic fungi from *Plachystachys lutea* Nees (Acanthaceae), an ornamental plant native to South America. Based on multilocus sequence analyses, *Diaporthe* species were the highest isolated. Meanwhile, other species such as *Nemania, Xylaria, Phyllosticta*, and *Alternaria* represented a small fraction of the strains. One advantage of these particular endophytic fungi is their ability to produce spores, a particular characteristic of the greatest potential inoculant since those that do not produce spores have limited large-scale application in the field (Murphy et al., 2018).

Regarding the growth-promoting abilities of endophytes, this work delineates details of plant growth promotion assays by endophytic fungi isolated from *P. lutea*. Phosphate solubilization, IAA production, antagonistic activity, and greenhouse experiments by the endophytes was investigated.

Material and methods

Microorganisms

There were four endophytic fungi (*Nemania* sp. PL27, *Xylaria berteroi* PL36, *Phyllosticta capitalensis* PL 49, and *Alternaria* sp. PL75), isolated from healthy leaves of *P. lutea*, employed in this work. The previous isolation was performed by leaf fragmentation done by Ribeiro et al. (2018). These endophytes were retrieved from the Collection of Endophytic and Environmental Microorganisms (CMEA) from the Laboratory of Microbial Biotechnology, Department of Biotechnology, Genetics and Cell Biology, *Universidade Estadual de Maringá* in Brazil.

The fungi pathogens *Fusarium oxysporum* (ATCC2163, André Tosello Foundation, Campinas, state São Paulo, Brazil), *Alternaria* sp. (CNPUV674, Embrapa Grape and Wine, Bento Gonçalves, state Rio Grande do Sul, Brazil), *Glomeralla* sp. (CNPUV378, Embrapa Grape and Wine, Bento Gonçalves, state Rio Grande do Sul, Brazil), *Colletotrichum* sp., *Dydimella bryoniae, Moniliophthora perniciosa*, and *Fusarium solani* (Department of Genetics College of Agriculture Luiz de Queiroz [ESALQ/USP] Piracicaba - Brazil) were used in dual culture assays.

Phosphate solubilization

The fungal solubilization efficiency was carried out using a modified Pikovskaya's agar medium (Nopparat, Jatupornpipat, & Rittiboon, 2009), composed of $(NH_4)_2SO_4$ (0.5 g), KCL (0.2 g), $MgSO_4.H_2O$ (0.1 g), $MnSO_4.H_2O$ (0.004 g), NaCl (0.2 g), D-Glucose (10 g), $FeSO_4.7H_2O$ (0.002 g, Sigma-Aldrich), yeast extract (0.5 g, Kasavi), agar (18 g), and distilled water (900 mL). The phosphate solution was prepared separately and included xanthan gum ($C_{35}H_{49}O_{29}$, 0.5 g), calcium phosphate [$Ca_3(PO_4)_2$, 0.5 g], and distilled water (100 mL). After the solutions were autoclaved, they were mixed and poured into the Petri dishes. A mycelial plug (6 mm diameter) of seven-day-old cultures of each endophyte was inoculated into the centre of the plate and incubated at 28ºC for six days. All the assays were carried out in triplicate, and the halo zone and the diameter of fungal growth was measured each day. The phosphate solubilization capability of the fungi was analyzed according to the equation described by Pande, Pandey, Mehra, Singh, and Kaushik (2017): (fungus diameter + halo zone)/ fungus diameter.

Fungal indole acetic acid production

Mycelia plugs of endophytes were cultured in 10% Potato Dextrose Broth medium (Neogen, Lansing, USA) supplemented with L-tryptophan (5 mM) (Sigma-Aldrich, state São Paulo, Brazil), and incubated in the dark at 28ºC for seven days. Then, fungal mycelia were separated using centrifugation for 5 min. at 15 000 x g. The supernatant (1 mL) were collected, and 2 mL of Salkowski's reagent was added, and this mixture was incubated for 30 min. The fungal-produced IAA was estimated by reading color intensity in a spectrophotometer at 520 nm, and the amount of production was calculated following a standard graph prepared using indole-3-acetic acid commercial (Sigma-Aldrich, state São Paulo, Brazil).

In vitro antagonistic activity

The antagonistic activity of strain *Alternaria* sp. PL75 was evaluated against seven phytopathogens using the paired-culture technique. Meanwhile, the metabolic extract obtained with ethyl acetate was evaluated by agar diffusion test.

Metabolic extraction from the fermented culture and *in vitro* antagonism assays were carried out following the protocol described by Polonio et al. (2015). For the agar diffusion test, Potato Dextrose Agar plates received 5 mm autoclaved filter paper plugs inoculated with 10 µL of metabolites (70 mg mL^{-1}) in the opposite position of phytopathogens plugs (6 mm diameter), and were incubated at 28ºC for seven days. For the negative control, the paper plugs received 10 µL of methanol. Competitive interactions between endophytes and phytopathogens were determined according to the rating scale proposed by Ribeiro et al. (2018).

Evaluation an endophyte for plant growth promotion in tomato seedlings

A Greenhouse experiment was performed at the Department of Agronomy at the *Universidade Estadual de Maringá*, state Paraná, Brazil. Santa Clara's tomato seeds were surface-disinfected by immersing in 70 ethanol (2 min.), followed by 1 sodium hypochlorite (3 min.), and 70% ethanol (30 s), and then seeds were rinsed with autoclaved distilled water ten times.

A seven-day-old culture (600 mm diameter) of *Alternaria* sp. PL75 was macerated in 80 mL of autoclaved distilled water, and then, the mixture was shaken in a vortex. Untreated control plants (T1), plants applied immediately of endophyte suspension (T2), and plants treated with endophyte suspension while transplanted (T3), were used in the experimental setup. The seeds were germinated in plastic trays containing autoclaved (at 121°C for 60 min.) Mecplant substrate (composed by pine bark, vermiculite, pH soil amendment, macro, and micronutrients). Twelve seeds in plastic trays received 1 mL of endophyte suspension and were maintained 35 days in a greenhouse under ambient natural conditions (T2).

After attaining 35 days of growth, all plants were transplanted in four plastic pots (three seedlings per potting) containing 2.8 L of the potting mixture (autoclaved soil and substrate in the ratio 1:1, v:v). During the transplantation, a few plants received 1 mL of endophyte suspension (T3). Control seedlings did not receive any endophyte suspension (T1). These plants were maintained under the greenhouse conditions described above. Plants were sprayed with water eight times per day for 15 min. until the end of the experiment.

The biometric parameters were evaluated 68 days after potting and before the reproductive process. We analyzed the number of leaves, root length (cm), shoot length (cm), dry and humid weight (g) of the shoot, and root biomass for each treatment. The shoot length was measured to the terminal leaf, and the biomass weight of the shoot and root were recorded after 120 hours (at 60ºC) of drying. The ratio between the root and shoot length was calculated by dividing the dry weight of the root by the dry weight the shoot.

The effect of *Alternaria* sp. PL75 on growing tomato seedlings was assessed by comparing (T1) untreated plants and (T2) plants treated with 1 mL endophyte suspension.

On the eighth day after inoculation, the first count of germination was recorded by analyzing the number of healthy seedlings. After seven days, the final count was performed, obtaining the percentage of germination.

The Emergency speed index was calculated according to the Equation 1:

$$EVI = E1/N1+E2/N2+...En/Nn \quad (1)$$

where:

EVI = Emergency speed index; E1, E2, En = the number of plantlets germinated at day; and N1, N2, Nn = first count after potting until the final count after potting.

Statistical analysis

The phosphate solubilization assay was statistically analyzed using a mixed-effects model. This model is based on the fixed effects of time and strain, the interaction between them, and a random effect for plates. Multiple comparisons test was performed in R statistical software (R Core Team, 2018) comparing the rates by Tukey test ($p < 0.05$). Antagonism and plant growth traits rates were compared by using the Scott-Knott method ($p < 0.05$) in the statistical program Sisvar 5.5 (Ferreira, 2011).

Results and discussion

Phosphate solubilization

On the first day, the strain *Alternaria* sp. PL75 was the only one that showed a zone of clearance around the fungal colony (2.57 cm). Other fungi exhibited halo zone formation on the second day. On the sixth day of incubation, *Alternaria* sp. PL75 and *Phyllosticta capitalensis* PL49 showed positive results with 4.29 and 3.91 cm, respectively. Although *Nemania* sp. PL27 (2.66 cm) and *Xylaria berteroi* PL36 (2.71 cm) had shown the halo zone on the second day, there was a decrease in their solubilization activity (Table 1).

There was a significant difference between *Nemania* sp. PL27 and *X. berteroi* PL36 in relation to *Alternaria* sp. PL75, regarding the halo zone rate on the third day. We also observed a statistically significant difference between *X. berteroi* PL36 and *P. capitalensis* PL49. Although *Alternaria* sp. PL75 demonstrated the highest rate for phosphate solubilization, there was no significant difference in comparison to *P. capitalensis* PL49 over time. Nevertheless, *Alternaria* sp. PL75 and *P. capitalensis* PL49 showed strong results after the third day (Table 1).

Table 1. Phosphate solubilizing activity of endophytic fungi isolated from *Pachystachys lutea*.

Endophytes	Phosphate solubilization halo zone				
	2 days	3 days	4 days	5 days	6 days
PL27 *Nemania* sp.	2.66 (0.20)^{a}	2.62 (0.15)^{ac}	2.39 (0.04)^{a}	2.24 (0.02)^{a}	2.18 (0.04)^{a}
PL36 *Xylaria berteroi*	2.71 (0.15)^{a}	2.28 (0.17)^{a}	2.32 (0.14)^{a}	2.47 (0.21)^{a}	2.60 (0.15)^{a}
PL49 *Phyllosticta capitalensis*	2.82 (0.54)^{a}	3.17 (0.53)^{bc}	3.44 (0.33)^{b}	3.81 (0.36)^{b}	3.91 (0.32)^{b}
PL75 *Alternaria* sp.	3.04 (0.30)^{a}	3.38 (0.23)^{b}	3.61 (0.00)^{b}	3.99 (0.29)^{b}	4.29 (0.35)^{b}

Tukey's test ($p < 0.05$), comparison day-to-day between the strains. Values in the parentheses reflect standard deviation. Averages followed by the same letter are not statistically different according to Tukey's test, at $p < 0.05$.

IAA production

The fungi *Nemania* sp. PL27 and *Alternaria* sp. PL75 were able to produce IAA according to their absorbance measurements. Based on the standard curve prepared with commercial indole-3-acetic acid ($R^2 = 0.99$), *Alternaria* sp. PL75 produced 1.73 and *Nemania* sp. PL27 amounting 1.86 µg mL^{-1} IAA.

In vitro antagonistic activity

Considering the amount of IAA produced and inorganic phosphorus solubilization by *Alternaria* sp. PL75, this strain was chosen for other assays including *in vitro* antagonism and greenhouse experiments.

According to the ANOVA, significant results were observed in dual-culture assays and agar diffusion tests against the phytopathogens. Table 2 shows the inhibition index percentage and competitive interactions between *Alternaria* sp. PL75 and the pathogens. We've also summarized the results of the efficiency antifungal activity of the metabolic extract in Table 2.

Table 2. Antagonism assay of endophytic fungus *Alternaria* sp. PL75 isolated from *Pachystachys lutea* against phytopathogens. The average area of mycelial growth of phytopathogens in cm^2 (A), percentage of inhibition rate (IP%), and Competitive interactions (CI).

Phytopathogens	Treatment					Control	
	Endophyte			Crude Extract			
	A	IP%	CI	A	IP%	Methanol	Only Pathogen
Fusarium oxysporum	33.63^{c}	20.68	A	25.18^{d}	46.04	46.66^{a}	42.40^{b}
Alternaria sp.	30.83^{b}	25.19	A	24.15^{c}	39.73	40.07^{a}	41.21^{a}
Colletotrichum sp	43.42^{b}	11.41	DA1	36.80^{c}	24.36	48.65^{a}	49.01^{a}
Fusarium solani	26.50^{c}	21.97	A	30.30^{b}	11.17	34.11^{a}	33.96^{a}
Moniliophthora perniciosa	31.30^{c}	52.11	A	20.02^{d}	64.14	55.83^{b}	65.36^{a}
Glomeralla sp.	32.12^{a}	0.00	A	20.32^{b}	35.84	31.67^{a}	31.22^{a}
Dydimella bryoniae	11.05^{b}	53.18	-	22.16^{a}	12.13	25.22^{a}	23.60^{a}

Scott-Knott test ($p < 0.05$) made for each pathogen separately, average followed by the same letter are not statistically different. Competitive Interaction: A – deadlock with mycelial contact; DA1 – Partial replacement pathogen growth on the endophyte after initial deadlock with mycelial contact.

The highest levels of inhibition were observed against *Moniliophthora perniciosa*. The crude extract showed a percentage of inhibition rate of 64.14 and the endophyte inhibition 52.11%, both of which were significantly higher when compared to their controls. We observed a high rate of inhibition for *Fusarium oxysporum* by endophyte *Alternaria* sp. PL75 and its crude extract of secondary metabolites; however, the activity of the

fungal extract was higher (46.04%) than the endophyte (20.68%). We also observed the same results against the pathogens *Alternaria* sp. (25.19%) and *Colletotrichum* sp. (11.41%) when compared to the antagonistic activity from the endophyte and its extract (39.73 and 24.36%, respectively) (Table 2). The endophyte antagonism rate (21.97%) was found to be the most efficient in inhibition the mycelial growth of the phytopathogen *Fusarium solani* than the endophyte's crude extract (11.17%).

Although we did not see antagonistic action against *Glomerella* sp., the secondary metabolites produced by the endophyte showed statistical significance in the agar diffusion test against the pathogen (35.84%). For *Didymella bryoniae*, we only observed inhibition for paired-culture assay (53.18%), however, due to irregular colony growth of the pathogen, we were unable to define the competitive interaction (Table 2).

The competitive interaction of type A was dominant (inhibition with mycelial contact). Partial pathogen growth on the endophyte after initial inhibition with mycelial contact (DA1) was found against *Colletotrichum* sp., this implies that the endophyte *Alternaria* sp. PL75 is less effective at controlling the growth of this pathogen. Despite the type A competitive interactions that were observed against *Glomerella* sp., the endophyte was not able to reduce its mycelial growth. We hypothesized that there could be, in this case, an overgrowth of the pathogen on the endophyte.

Evaluation of plant growth-promoting

There were no symptoms in plants inoculated with the endophyte, which suggests that there was no apparent damage caused during the experiment. The endophyte *Alternaria* sp. PL75 enhanced the EVI of the tomato seedlings treated (18.46) compared to the non inoculated control plants (16.56), according to Tukey's test at a significance level of 0.05. The percentage of germination for control plants was 60% attained fifteen days after potting; meanwhile, the percentage for treated plants (T2) was 81.63%. These results showed a strong increase in plantlets' germination induced by the fungal suspension without modifying their development.

There were significant differences in the shoot length. Plants treated with fungal suspension after 35 days (T3) showed the highest increase (55.38 mm), followed by T2 plants (52.31 cm) when compared to controls (41.67 cm) (Table 3, Figure 1).

The ratio between the root and shoot length in T3 was significantly higher (0.37) when compared with the control (0.22) and T2 (0.25). The increase of this ratio suggests an overproduction of the root compared to the shoot (Table 3).

In the present study, all strains of endophytic fungi showed phosphate solubilization activity. Pande et al. (2017) evaluated the solubilization of phosphate by endophytic bacteria and described high solubilization activities, ranging from 4.48 to 4,88 during seven days of incubation. This was similar to the results found herein, where *Alternaria* sp. PL75 exhibited a phosphate solubilization value of 4.29 on the sixth day, suggesting strong activity.

Table 3. Evaluation of biometric parameters in the greenhouse experiment with tomato seedlings with and without *Alternaria* sp. PL75.

Biometric Parameters	Treatments		
	Treatment 1	Treatment 2	Treatment 3
Number of leaves	8.83 (0.98)[a]	8.5 (0.76)[a]	8.88 (2.53)[a]
Shoot length (cm)	41.67 (7.89)[a]	52.31 (8.39)[b]	55.38 (9.16)[b]
Root length (cm)	43.83 (11.37)[a]	37.25 (12.02)[a]	39.13 (6.66)[a]
Shoot humid biomass (g)	39.51 (16.86)[a]	52.11 (12.58)[a]	51.28 (22.55)[a]
Root humid biomass (g)	7.66 (3.54)[a]	8.60 (3.02)[a]	13.41 (7.21)[a]
Shoot dry biomass (g)	5.21 (2.18)[a]	5.44 (1.28)[a]	6.44 (3.15)[a]
Root dry biomass (g)	1.12 (0.66)[a]	1.44 (0.88)[a]	2.37 (1.35)[a]
Ratio root: shoot	0.22 (0.06)[a]	0.25 (0.11)[a]	0.37 (0.13)[b]

Average followed by the same letter does not differ by Scott-Knott test ($p < 0.05$). Values in the parentheses reflect standard deviation. Treatment 1: untreated control plants; Treatment 2: seeds received an endophyte *Alternaria* sp. PL75 suspension inoculum; Treatment 3: plants treated with endophyte suspension while transplanted after 35 days of growth.

Next to nitrogen, phosphorus is an essential element required for plant development, growth, survival, and protein synthesis. Agricultural production is determined by the amount of free phosphorus available in the soil (Pande et al., 2017; Santos et al., 2018). In the soil, most of the phosphorus is in organic matter or mineral form, and it is absorbed by plants as phosphates. Nonetheless, it should be noted that phosphorus in the soil could associate with other minerals that make it unavailable to be absorbed by plants, even in higher concentrations (Valetti, Iriarte, & Fabra, 2018).

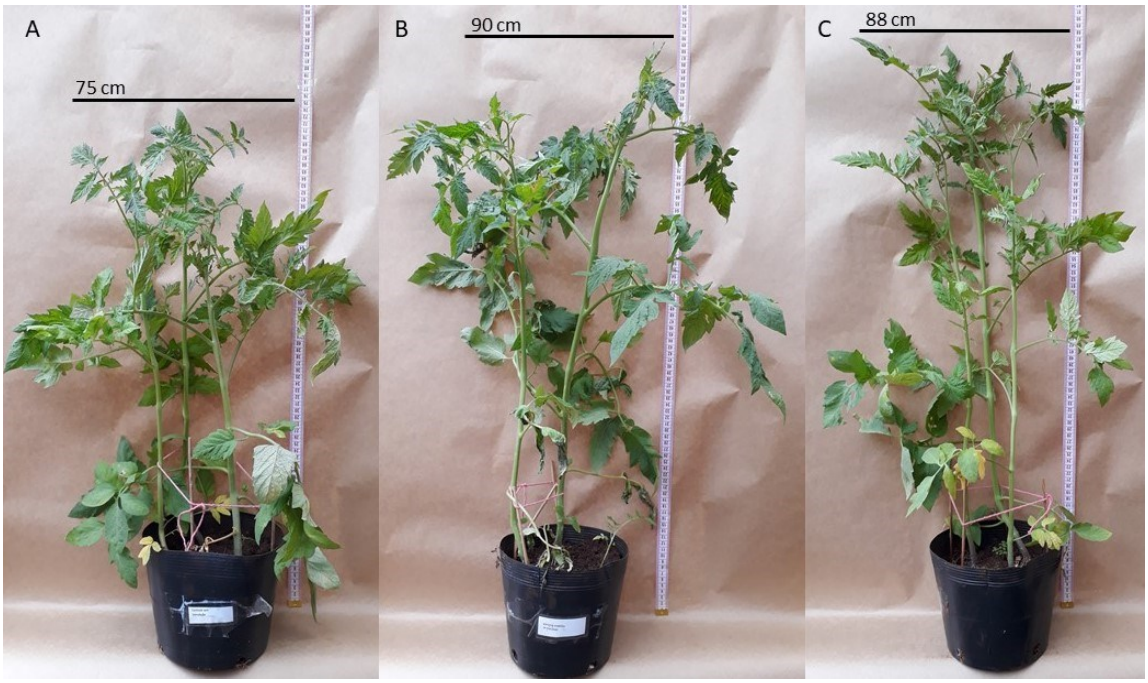

Figure 1. A: Treatment 1 with untreated control plants; B: Treatment 2 with endophyte *Alternaria* sp. PL75 suspension inoculation of seeds; C: Treatment 3 with plants treated by endophyte suspension while transplanted after 35 days of growth.

Microorganisms-mediated phosphate solubilization has great importance in the agriculture field, because this process plays an essential role in promoting plant growth and improving agriculture productivity. There has been an increased focus on applications of these microorganisms in the crop field to better understand host-microbe interactions (Valetti et al., 2018; Adhikari & Pandey, 2019).

There are many processes involved in the microorganism's ability to solubilize phosphate, such as enzyme production (i.e., phosphatases and phytases) and organic acids (Pande et al., 2017; Adhikari & Pandey, 2019). Different phosphate sources, such as calcium phosphate, iron, and aluminium, can be solubilized by microorganisms. However, higher solubilization has been shown employing calcium phosphate (Spagnoletti, Tobar, Pardo, Chiocchio, & Lavado, 2016; Adhikari & Pandey, 2019).

We also evaluated fungal-produced IAA, and both *Nemania* sp. PL27 and *Alternaria* sp. PL75 showed positive results. Indole acetic acid is an auxin in plants that regulate growth, cell division, and differentiation process (Yu, Yu, Fan, Wang, & Liu, 2016). Endophytic microbes are strong IAA producers, Yu et al. (2016), studied 396 endophytic bacteria isolated from soybean and corn plants, and reporting that 39.6% of the strains were able to produce IAA ranging from 1 to 23 μg mL^{-1}. Moreover, 14 of those isolates showed growth-promoting abilities in soy and wheat plants.

The amount of IAA-produced can be modulated by the incubation process, tryptophan supplementation, and analysis methods (Yu et al., 2016). Shah, Shrestha, Maharjan, Selosse, & Pant (2019), reported endophytic fungi isolated from *Dendrobium moniliforme* produced excess IAA when the medium was supplemented with L-tryptophan. It has been noted that crop-associated endophytes have the potential to serve as a promising biotechnological tool for plant growth promotion, and to help its hosts endure environmental and biological stress conditions. Thus, auxins produced by these microorganisms could be an important regulator for plant development, especially under stress conditions (Ikram et al., 2018; Strobel, 2018).

Regarding the antagonism assay against the phytopathogens, *Alternaria* sp. PL75 and its metabolic crude extract showed inhibition against the pathogens, and the most inhibition was observed against *Moniliophthora perniciosa,* the causal agent of witches' broom disease of cacao. The application of endophytes to serve as biological control agents has been increasing. It is believed that these microorganisms can protect against pathogens or insect attacks (Strobel, 2018; Yan et al., 2019).

Endophytes may reduce the incidence or severity of plant diseases by contending for nutrients, antagonizing phytopathogens (e.g., mycoparasitism), producing a range of antimicrobial compounds, or triggering induced systemic resistance. Just the fact these microorganisms colonize and mobilize nutrients to their hosts, it would avoid pathogenic microbial infection (Ab Rahman, Singh, Pieterse, & Schenk, 2018; Nandhini et al., 2018; Ribeiro et al., 2018; Silva et al., 2019).

We hypothesized that the type A competitive interaction found herein, reflects competition for colonization. Further, the antagonistic activity exhibited against *M. perniciosa* and *F. oxysporum* by the fungal extract of *Alternaria* sp. PL75, suggests the endophyte produced antifungal compounds. Secondary metabolites play a crucial role in maintaining the balance between endophytes, competitors, and plant's hosts (Silva et al., 2019).

Alternaria species have been described as an endophyte, a saprophyte, and a pathogen of a range of crops worldwide including grapes, wheat, tomatoes, potatoes, and others (Specian et al., 2016; Somma, Amatulli, Masiello, Moretti, & Logrieco, 2019). Nonetheless, many of these fungi are usually plant pathogens, but they can also be isolated as endophyte, without causing any damage to their hosts (Strobel, 2018). Similar to what was presented in this work, *Alternaria* sp. PL75 did not trigger any symptoms of disease.

It has been reported that, according to the niche, endophytic and pathogenic genomes are differentiated in gene expression or repression. There is an evolutionary transition from a pathogenic to an endophytic lifestyle when genes linked to secondary metabolites are activated and genes associated with pathogenicity are repressed (Hacquard et al., 2016; Nandhini et al., 2018).

Besides, it was observed that inoculating tomato seeds with *Alternaria* sp. PL75 increased the percentage of germination. Similar results were obtained by Nandhini et al. (2018), in which millet plants had their germination and growth augmented by endophytes that produce IAA.

It was demonstrated that endophytic microorganisms could improve seed germination and take in nutrients, thereby playing an essential role in promoting plant growth (Vujanovic & Vujanovic, 2007; Rai et al., 2014; Shah et al., 2019). During the germination process, endophytic fungi degrade the cellulose present in the cuticle, which makes carbon available to plantlets and improves germination (Rai et al., 2014).

Our results indicated an improvement in shoot length of tomato plants treated with an endophyte suspension. Shah et al. (2019) also evaluated the growth-promoting ability by endophytic fungi. These authors reported a significant improvement in *Rhynchostylis retusa* plantlets by supplementing the culture medium with fungi. Valetti et al. (2018) performed another investigation with endophytes isolated from *Brassica napus,* which were able to solubilize phosphate, and showed strong results in greenhouse experiments, especially in shoot length.

Endophyte-mediated growth promotion may result from improved nutrition mediated through the increased uptake and availability of nutrients in the soil (i.e., phosphorous), phytohormones production (i.e., IAA), and the ability of endophytes to help their host adapt under abiotic and biotic stress (Jogaiah, Abdelrahman, Tran, & Shin-Ichi, 2013; Rai et al., 2014).

Conclusion

The endophytic strain *Alternaria* sp. PL75 showed the best results in phosphate solubilization, IAA production, and in *in vitro* dual culture assays against pathogenic fungi. The endophyte improved the emergence and germination of tomato seeds, and also led to an increase in shoot length. We believe that this might be correlated with the phosphate-solubilizing ability, which increases soil fertility and IAA production. In summary, this strain displayed important characteristics in the biological control of phytopathogens. Furthermore, it served as a growth-promoting agent, suggesting its useful application in the field as a biofertilizer to contribute to friendly agriculture. Further studies are needed to evaluate the application of *Alternaria* sp. PL75 in other crops of agricultural interest, and in the field to evaluate the results on a large scale.

Acknowledgements

The authors would like to thank Capes (*Coordenação de Aperfeiçoamento de Pessoal de Nível Superior*) for the Doctoral's scholarship and Capes/PNPD-UEM post-doctoral scholarship and CNPq (*Conselho Nacional de Desenvolvimento Científico e Tecnológico* - 307603/2017-2) and the Seti-UGF (TC n.65/2018) for financial support.

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