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BIOTECHNOLOGICAL POTENTIAL OF PURPLE PASSION FRUIT ENDOPHYTIC FUNGI (*Passiflora edulis* f. *edulis*; Passifloraceae)

Potencial biotecnológico de hongos endófitos de gulupa (*Passiflora edulis* f. *edulis*; Passifloraceae)

Stephany HURTADO-CLOPATOSKY[1,2*], Luz Marina MELGAREJO[1], Ginna Natalia CRUZ-CASTIBLANCO[3], Lilliana María HOYOS-CARVAJAL[4]

[1.] Laboratorio de fisiología y bioquímica vegetal, Facultad de Ciencias, Departamento de Biología, Universidad Nacional de Colombia. Bogotá, Carrera 30 con calle 45, shurtadoc@unal.edu.co, lmmelgarejom@unal.edu.co

[2.] Facultad de ciencias Agropecuarias, Universidad de Cundinamarca, Diagonal 18 N° 20 - 29, Fusagasugá, Colombia, shurtadoc@ucundinamarca.edu.co

[3.] Corporación Colombiana de Investigación Agropecuaria Agrosavia, Km 14 Vía Mosquera - Bogotá, Colombia, gcruz@agrosavia.co.

[4.] Facultad de Ciencias, Departamento de ciencias Agrícolas, Universidad Nacional de Colombia, Carrera 65 No. 59A - 110 Bloque 11 - Oficina 102, Medellín, Colombia, limhoyosca@unal.edu.co.

** For correspondence: shurtadoc@unal.edu.co,shurtadoc@ucundinamarca.edu.co*

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ABSTRACT

There is evidence that all plants coexist with endophytes, indicating a fundamental role that is unknown. The objective was to evaluate the biotechnological potential of endophytes from *Passiflora edulis* f. *edulis* in two aspects: as a plant growth promoter and as a control for the pathogen *Fusarium oxysporum*. An *in vitro* phase was used in which the antifungal activity in a dual culture was studied, where the UNE075 and UNE004 fungi inhibited the radial growth of the pathogen by up to 67.63 % and 63.89 %, respectively. Additionally, higher inhibition percentages were seen with UNE075 (33.78 %) and UNE098 (32.32 %) because of the action of volatile organic compounds on *F. oxysporum*. Likewise, the production of indoleacetic acid (IAA) as a growth-promoting compound was quantified, with notable results with UNE017 (11.99 µg/mL) and UNE022 (7.59 µg/mL). The capacity of the fungi to solubilize phosphorus was determined. UNE098 generated the greatest solubilization by reducing the pH culture medium. In the *in vivo* phase in the greenhouse, the effect of inoculation with endophytes on the growth of *P. edulis* f. *edulis* plants was evaluated. Biomass accumulation and leaf area were determined, where UNE067 stood out because of its effect on fresh weight, total dry weight. In general, the evaluated endophytes have biotechnological potential for use in organic crop management programs and for biological control.

Keywords: antifungal agent, biological control agent, endosymbionts, microbiota, phytopathogenic fungus.

RESUMEN

La evidencia muestra que todas las plantas conviven con endófitos, lo cual indica que desempeñan un papel fundamental aún por dilucidar. El objetivo de este trabajo fue evaluar el potencial biotecnológico de endófitos provenientes de *Passiflora edulis* f. *edulis*, en dos aspectos: como acción promotora de crecimiento vegetal y como controladores del patógeno *Fusarium oxysporum.* Se empleó una fase *in vitro* en la que se estudió la actividad antifúngica en cultivo dual, encontrándose que los hongos UNE075 y UNE004 lograron inhibir el crecimiento radial del patógeno hasta en un 67,63 % y 63,89 %, respectivamente. Adicionalmente, por acción de compuestos orgánicos volátiles sobre *F. oxysporum*, se observaron que los mayores porcentajes de inhibición fueron dados por los hongos UNE075 (33,78 %) y UNE098 (32,32 %). Asimismo, se cuantificó la producción de Acido indolacético (AIA) como compuesto promotor del crecimiento**,** destacándose UNE017 (11,99 µg/mL) y UNE022 (7,59 µg/mL). Por otra parte, se determinó la capacidad de los hongos para solubilizar el fósforo, UNE098 fue la cepa que generó mayor solubilización mediante la reducción

del pH en el medio de cultivo. En la fase *in vivo* en invernadero se evaluó el efecto de la inoculación con endófitos sobre el crecimiento de plantas de gulupa, se determinó la acumulación de biomasa, sobresaliendo UNE067 por su efecto sobre el peso fresco, el peso seco total y el área foliar. En general, se observó que los endófitos evaluados tienen potencial biotecnológico para uso en programas de manejo del cultivo orgánico y para control biológico.

Palabras clave: agente antifúngico, agente de control biológico, endosimbiontes, hongo fitopatógeno, microbiota.

INTRODUCTION

Purple passion fruit (gulupa) is a tropical plant with economic potential, which is increasing every year. For the 2023 period, growth reached 15 % (Forbes Staff, 2023). The main market is the Netherlands, which represents 83.67 % of the exported value. In Colombia, it is grown mainly in the departments of Cundinamarca, Boyacá, and Antioquia, with a production of 24.407 tons (Rincón and Ospino, 2021), which is mainly intended to supply the fresh fruit market where fruits with lower acidity and greater sweetness are preferred (de Jesus et al., 2023).

Considering the demands of the international market, fruits must meet a series of quality and safety standards that require the development of sustainable and environmentally friendly management alternatives (Echeverri, 2018; Guerrero-López et al., 2012; Rincón and Ospino, 2021).

The use of biotechnology makes it possible to take advantage of live microbes and their derivatives for the development of crop management strategies, reducing the use of synthetic agro-inputs that are dangerous for human health and the environment (Rojas Ramírez, 2013; Sánchez-Fernández et al., 2013). This is an alternative that can improve the limiting aspects of purple passion fruit cultivation, ensuring greater productivity and access to markets.

Fungi have been recognized for their ability to produce various compounds such as alkaloids, terpenoids, peptides, hydrocarbons, aromatic compounds, and other substances with biological activity and possible applications in the field of agriculture (Gupta and Sharma, 2020). In particular, the endophytes are of interest since they have a very close relationship with their host, spending part of their life cycle asymptomatically inside plants, establishing a relationship that is commonly symbiotic. Some are capable of synthesizing compounds that confer protection against biotic and abiotic factors and can also benefit their host by promoting growth (Oono et al., 2015; Sánchez-Fernández et al., 2013).

One of the strategies used by endophytes to promote plant growth is the production of auxins. Auxins are phytohormones that act at low concentrations in plants and are responsible for cell division, elongation, and differentiation; they also generate morphogenic processes such as embryonic development, formation of leaves, flowers, and lateral roots, and maintenance of the root meristem (Garay-Arroyo et al., 2014).

Endophytes also promote plant growth by making phosphorus available and maintaining its balance in the soil through different mechanisms, depending on the origin. Mineral phosphorus solubilization is mainly based on pH reduction (Patiño-Torres and Sanclemente-Reyes, 2014; Sharma et al., 2013). This characteristic is important because of the low availability of phosphorus in soils in tropical regions (Rojas Restrepo, 2015).

F. oxysporum is the causal agent of wilting in purple passion fruit and is a pathogenic fungus that colonizes the tissues of the cortex and xylem and prevents the movement of water and nutrients within plants, generating significant economic losses in crops (Ortiz and Hoyos-Carvajal, 2016). In addition, it is difficult to manage since it produces mycelium with rapid growth and resistance structures, such as chlamydospores, that impart a high capacity to survive in soil and plant tissues (Arbeláez-Torres, 2000; Rooney-Lathan et al., 2011).

It is important to integrate different management alternatives to prevent the development of wilt in purple passion fruit, including biological controls focused on the use of endophytes that take advantage of different mechanisms of action in these microbes. These mechanisms include induction of resistance, increases in the expression of defense genes, hyperparasitism, predation, and production of toxic compounds, antifungals, and volatile organic compounds (VOC) (Sánchez-Fernández et al., 2013; Wonglom et al., 2020; Zapata, 2019).

The objective was to evaluate possible biotechnological applications of endophytes for the management of purple passion fruit crops for promoting plant growth and biocontrol of the pathogen *F. oxysporum*.

MATERIALS AND METHODS

ENDOPHYTES

Nine endophytic fungi isolated from purple passion fruit plants (Hurtado, 2020) were used in the present study; their origin data are in Table 1. To isolate endophytic fungi, samples from different *P. edulis* f. *edulis* tissue were treated in a 1 % Cl_2 chamber for 3 minutes, then washed three times with sterile, distilled water. Four sample fragments, approximately 5 mm, were transferred to 12 Petri dishes, three for each culture medium. The media used for the isolation of the endophytes were PDA, eight vegetable juice agar (V8®), Sabouraud Agar (SA) and malt extract agar

(MA), added with Chloramphenicol 0.1 g/L. The tissue samples were placed in an incubator at 25 °C for between eight and ten days (Hurtado, 2020). Isolated endophytes were multiplied on a Potato Dextrose Agar (PDA) medium and incubated at 22°C in complete darkness for eight days before use in this study.

The taxonomic identification of the endophytes (Table 1) was carried out from the sequencing of ITS regions. The endophytes were sent to the Agrosavia corporation in Petri dishes for processing in an ABI 310GA device that uses an automatic capillary electrophoresis system. Sequencing was done with the Sanger methodology in a single forward direction. The results were compared with other ITS sequences available online, and the assembly was done on the NCBI BLAST platform.

INDOL ACETIC ACID PRODUCTION

From the respective endophytes, three disks of growing mycelium, 5 mm in diameter, were cultured in 10 mL of PDA medium enriched with 200 µg of tryptophan in Falcon tubes. They were incubated in the absence of light at 22°C for one week in a horizontal shaker at 150 rpm. Subsequently, they were centrifuged at 6000 rpm for 10 min at 4 °C. A 2 mL aliquot of the supernatant was removed, and 1 mL of Salkwoski's reagent (4.5 g $FeCl_3$/L in 10.8 M H_2SO_4) (Glickmann and Dessaux, 1995) was applied, left to stand for 30 min in dark room, and read at an optical density (OD) 540 nm. The medium without inoculation was used as the blank.

DETERMINATION OF PHOSPHORUS SOLUBILIZERS

The ability of endophytes to solubilize phosphorus was tested using the methodology described by Mehta and Nautiyal (2001), relating the solubilization capacity to the acidification of the medium by the microorganism of interest. The endophytes were inoculated in the same way but in 10 mL of NBRI medium with bromophenol blue (10 g Sucrose, 5 g $Ca_3(PO4)_2$, 2.5 g $MgCl_2*6H_2O$, 0.25 g $MgSO_4*7H_2O$, 0.2 g KCl, 0.1 g $(NH_4)_2SO_4$, 0.025 g Bromophenol blue, adjust to pH 7, and make up to a final volume of 1 L with distilled water). Subsequently, they were incubated for eight days in complete darkness at 20°C in a horizontal shaker at 150 rpm; then, they were centrifuged at 4000 g for 10 min, and 700 µL of the supernatant was removed for each sample, which was read at an optical density (OD) of 600 nm. The medium without inoculation was used as the blank (Gravel et al., 2007).

PLANT GROWTH PROMOTION BIOASSAY

This test was carried out under greenhouse conditions in the Biology Department of the National University of Colombia, Bogotá, at an altitude of 2625 meters above sea level, with an average temperature of 20.9°C and an average relative humidity of 59.21 %. *P. edulis* f. *edulis* plants were obtained from seeds, sown in sterile peat with nutrients for two months, when the substrate was removed, and the roots were washed with distilled water for subsequent inoculation with the respective endophyte.

For the preparation of the inocula, fragments of growing mycelium, 5 mm in diameter, were seeded in 15 Petri dishes with PDA medium and incubated for 13 days. To obtain the suspension, the methodology proposed by Golparyan et al. (2018) was followed. This suspension was filtered through sterile gauze and diluted in sterile, distilled water to a concentration of $1x10^6$ conidia/mL.

The inoculation of each endophyte was carried out with 20 mL/plant in ten plants with an inoculum suspension of $1x10^6$ conidia/mL using the root immersion technique for 2 min (Ortiz, 2012). This methodology was selected to favor the entry of endophytes since root transmission is a common form of endophyte recruitment in nature mainly in tropical areas (Garbeva et al., 2004; Medina et al., 2017). For non-inoculated plants (absolute control), the root was immersed in sterile, distilled water.

Each plant was transplanted into a pot with 300 g of sterile promix PGX® brand peat, composed of Canadian Sphagnum Peat (65 - 75 %), vermiculite, macronutrients, micronutrients, dolomitic and calcitic lime. To verify colonization, endophytes were re-isolated from samples of stem, leaves and roots from six plants for each treatment.

At the end of the trial, 63 days after inoculation, destructive sampling was carried out where the leaf area was calculated with ImageJ version 2015 using photographs and a tape measure as a size reference. The fresh weight of each organ and the total fresh weight were also determined. To estimate the dry weight, each sample was placed in a drying oven at 72 °C until constant weight.

IN VITRO ANTIFUNGAL ACTIVITY

The ability of endophytic fungi to inhibit the growth of a strain of the pathogen *F. oxysporum* obtained by Ortiz and Hoyos-Carvajal (2016) was tested. The dual plate culture methodology was used in a PDA medium with 5 mm mycelial growth discs of the pathogen and endophyte, which were located on opposite sides of the plate. For the control treatment, only the pathogen was cultured. The different mounts were kept in the dark at 25 °C ±2 °C for 13 days (Fernández-Barbosa and Suárez-Meza, 2009). Subsequently, the interaction was visually evaluated using the scale proposed by Badalyan et al. (2004), which describes three types of interactions: "Type A" corresponds to mutual inhibition or deadlock (growth of both fungi stops at the point of contact), "type B" is distance inhibition (growth of the pathogen stops without mycelial contact at a distance of at least 2mm), and "type C" is replacement

Table 1. Description the Strain, isolation conditions, and identification (by ITS regions) for fungal endophytes from purple passion fruit (*P. edulis* Sims f. *edulis*).

Strain	Coordinates		Origin site	Organ	Identification		
	Latitude (N)	Longitude (W)			Name	% Identity	% Coverage
UNE004				Root	*Trichoderma gamsii*	99.24	100
UNE010				Root	*Aspergillus wentii*	100	100
UNE017	5°37'47.0"	73°37'21.0"	Sutamarchán (Boyacá)	Stem	*Fusarium equiseti*	99.76	100
UNE022				Root	*Epicoccum nigrum*	99.76	100
UNE028				Stem	*Chaetomium globosum*	98.44	100
UNE063	4°17'56.0"	74°18'02.2"	Pasca (Cundinamarca)	flower	*Chaetomium globosum*	98.44	100
UNE067				Root	*Curvularia penniseti*	99.01	98
UNE075	5°37'47.0"	73°37'21.0"	Sutamarchán (Boyacá)	Fruit	*Trichoderma asperellum*	99.76	100
UNE098	4° 31' 51.27"	74° 20' 50.33	Granada (Cundinamarca)	Stem	*Trichoderma asperellum*	100	100

Source: Adapted from Hurtado, 2020.

or overgrowth (One of the fungi grows on the mycelium of the other, partially or totally). The growth radius of the pathogen was also measured, and the percentage of radial growth inhibition (PRGI) was calculated using the equation described by Ezziyyani et al., (2004).

$$PRGI=(R1-R2)/R1 * 100\ \%$$

Where, R1 represents the radial growth of the pathogen that grew in the control treatment in millimeters (mm), and R2 represents the radial growth of the pathogen that grew in the dual culture in mm.

This equation was also used to evaluate the capacity of endophytes to inhibit the growth of *F. oxysporum* by producing toxic, antifungal and VOC volatile organic compounds, following the methodology described by Li et al. (2018) and Wonglom et al. (2020), in which two Petri dish bases face each other to form a chamber. In this case, 60 x 15 mm plates were used with a PDA medium; then, the pathogen was inoculated in the center of one of the bases with a 5 mm diameter disc of mycelium, placing this plate at the top, and the endophyte was placed on the opposite base, forming the chamber that was hermetically sealed. The control consisted of a chamber where the pathogen grew alone. The cultures were placed in an incubator at 25°C for four days in the dark.

ANALYSIS OF RESULTS

For the data analysis, the assumptions of normality (Shapiro and Francia, 1972), independence of errors, and homogeneity of variance (Montgomery, 2004) were tested using the Shapiro-Wilk, Levene, and Durbin Watson test. A general and mixed linear model was proposed, where the fixed part of the model corresponded to the treatment, and the random part corresponded to the repetitions and plants. To find out if there are significant differences between isolates, multiple comparisons of the variables were performed with the LSD Fisher mean comparison test with a confidence level of ($p<0.05$) and the Bonferroni correction. The evaluated variables were: "PRGI *F. oxysporum* from the interaction in dual culture with endophytic fungi", PRGI *F. oxysporum* from exposure to VOCs produced by different endophytic fungi, IAA, phosphorus solubilizers, fresh weight, dry weight, and leaf area. The data were analyzed using R statistical software and Infostat 2019.

RESULTS

INDOL ACETIC ACID PRODUCTION

The production of IAA by endophytes is of interest because it is an important regulator of plant growth and is related to the development of adventitious roots, improving the absorption of water and nutrients. Table 2 presents the values of the capacity of the endophytes to produce IAA and other related compounds in the same detection spectrum. UNE017 showed the highest production, releasing an average of 11.99 µg/mL into the medium, followed by UNE022 (7.59 µg/mL) and UNE004 (4.54 µg/mL). The other endophytes produce low amounts of IAA, with averages of 3.45 to 0.26 µg/mL.

PHOSPHORUS SOLUBILIZERS

According to the results (Table 2), there were significant differences in the ability of endophytes to solubilize phosphorus. The UNE098 strain produced the highest acidification of the medium as a mechanism to solubilize inorganic phosphorus, followed by UNE017 and UNE010, which also solubilized phosphorus but at a smaller quantity. UNE004, UNE028 and UNE075 did not produce changes in the color of the medium, indicating low or null production of organic acids.

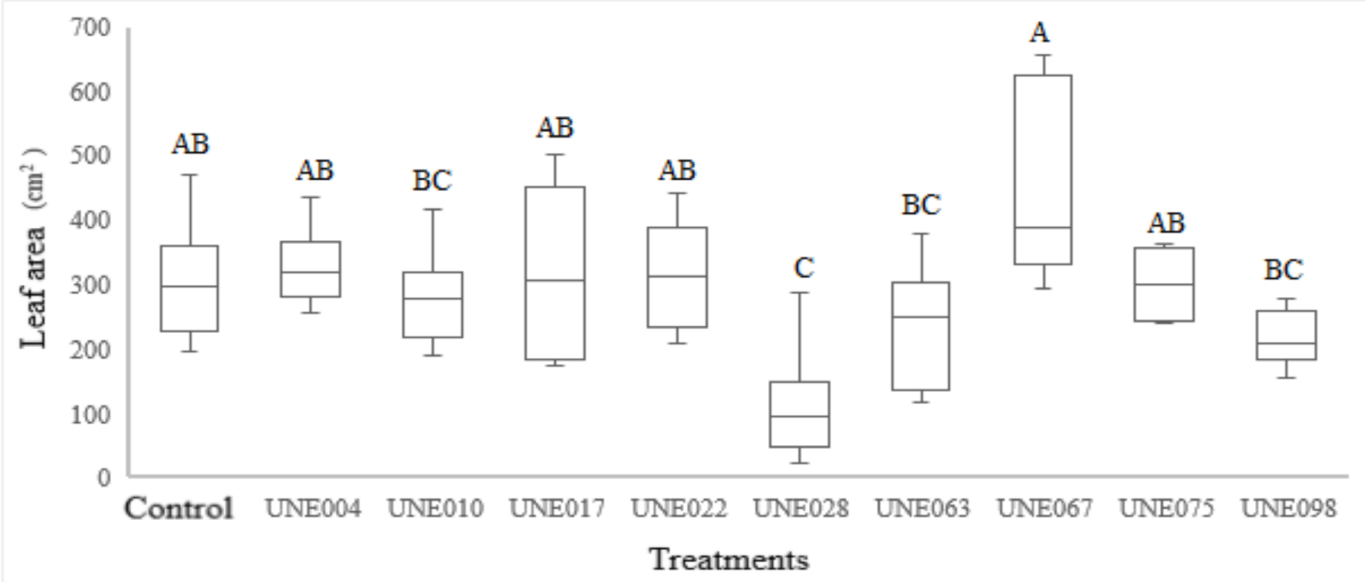

Figure 1. Leaf area of gulupa plants (*P. edulis* Sims f. *edulis*) at 63 days after inoculation with endophytic fungi. Non-inoculated plants (control). Means with the same letter are not significantly different (LSD Fisher p > 0.05), for six replicates; bars represent standard deviation.

BIOMASS

Table 3 shows the biomass accumulation behavior of the purple passion fruit plants inoculated with the respective endophytic fungi. The plants with more accumulation of biomass were inoculated with endophyte UNE067 in all parts (leaves, stem, root), including the total weight. On the contrary, the plants exposed to UNE028 had lower values for all variables for the accumulation of fresh and dry biomass. On the other hand, the treatments with UNE010, UNE063 and UNE098 generated a lower accumulation of fresh weight with respect to the non-inoculated plants in the leaves, stems and total weight.

FOLIAR AREA

The plants inoculated with the endophytes UNE004, UNE017, UNE022 and UNE075 had similar behaviors for leaf area with respect to the control plants without inoculation (Fig 1), which indicates that inoculation with these endophytes did not affect this variable. The plants inoculated with the endophyte UNE028 had the smallest leaf area, presenting a restriction in the growth of the leaves. The plants inoculated with UNE067 had higher values for leaf area (Fig 1).

ANTIBIOSIS IN DUAL CULTURE

The antifungal activity of the endophytes was studied (table 2), and it was found that all strains limited the radial growth of the pathogen *F. oxysporum*, by between 20.95 % and 67.63 %. UNE075 had the greatest antifungal effect with a PRGI of 67.63 %, followed by the UNE004 with a similar capacity, achieving 63.89 % inhibition. UNE067, UNE028, UNE098 and UNE063 had average values between 52.16 % and 58.99 %. Finally, the UNE017, UNE010 and UNE022 strains achieved lower inhibition averages with 37.5 %, 27.47 % and 20.95 %, respectively.

Each endophyte presented more than one type of interaction (Fig 2) according to the scale proposed by Badalyan et al. (2004). The strains UNE010, UNE017, UNE028, UNE063, and UNE067 presented a "type A" interaction. The strains UNE004, UNE017, UNE063, UNE075, and UNE098 presented a "type C" interaction. The UNE010 and UNE022 strains presented a "type B" interaction.

ANTIBIOSIS BY VOC ACTION

UNE004, UNE010, UNE022, UNE067, and UNE063 generated very low inhibition of the radial growth of *F. oxysporum*, with averages between 1.25 % and 3.79 %. UNE017 and UNE028 produced moderate inhibition, between 10.46 % and 12.46 %. Finally, the endophytes UNE075 and UNE098 produced greater inhibition of the radial growth of *F. oxysporum* through the production of VOC, between 33.78 % and 32.32 %, respectively (Table 2).

DISCUSSION

Endophytic fungi develop bioactive secondary metabolites that give them the ability to protect their host plant and thus successfully thrive within it since they play an important role as growth promoters, antagonists, and regulators of the

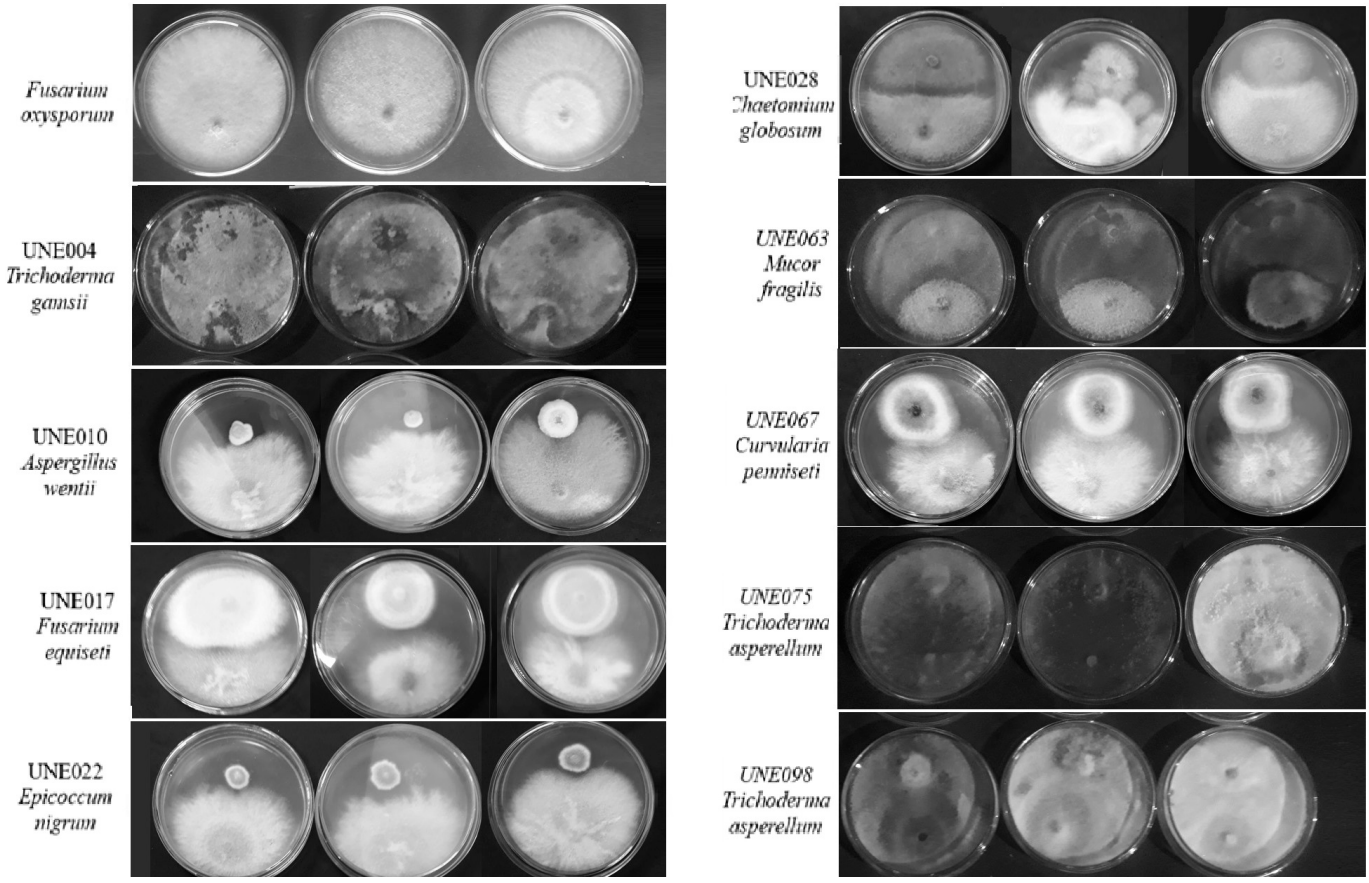

Figure 2. Antagonism of nine endophytes from purple passion fruit (*P. edulis* Sims f. *edulis*) against the pathogen *F. oxysporum* in *vitro*.

environment (Wang et al., 2023; Espinosa-Zaragoza et al., 2021; Sánchez-Fernández et al., 2013).

This study determined whether endophytes from *P. edulis* Sims f. *edulis* could produce 3-indole acetic acid and related products, where UNE017 produced 11.99 µg/mL; according to Ríos et al., (2016), this value is high for endophytic microorganisms that produce IAA.

The production of IAA by a microorganism can vary widely depending on several factors, such as the amount of the precursor L-tryptophan in the substrate, the temperature, the incubation period, the growth phase of the microorganism and, in general, when faced with different conditions such as growing in an *in vitro* medium or inside a plant (Numponsak et al., 2018). However, if the culture conditions that stimulate the production of IAA are optimized and its concentration is standardized at biologically active levels in the plant, the endophytes UNE017, UNE022 and UNE004 could be used for the development of bioproducts (Lara et al., 2011; Numponsak et al., 2018).

There was no relationship between the accumulation of biomass or leaf area with the production of IAA *in vitro* in the endophytes. It has been reported that the response of plants is different between tissues, stages of development and concentrations of IAA, so determining the role of this phytohormone in development is complex (Garay-Arroyo et al., 2014). It has been reported that the effect of inoculation with IAA-producing endophytes on plant growth may occur in the first days and not be maintained over time. Likewise, the response to very high levels of IAA of different plant species can vary between stimulating foliar and root growth to causing negative effects on development such as root growth inhibition (Arango et al., 2012).

This study determined the ability of *P. edulis* f. *edulis* endophytes to solubilize mineral phosphorus. UNE017 and UNE098 presented a higher solubilization capacity by reducing the pH of the medium. Therefore, these strains have high potential to improve plant nutrition when phosphorus is limiting in the substrate (Mehta and Nautiyal, 2001), which is why several authors relate this capacity to plant growth promotion (Angulo et al., 2014; Corrales et al., 2014; Gravel et al., 2007; Mehta and Nautiyal, 2001).

The use of phosphorus-solubilizing microorganisms to improve the availability of phosphorus makes it possible to reduce the use chemically synthesized fertilizers. Adesemoye et. al (2009) managed to reduce the contribution of phosphorous fertilizer by 25 % by using inoculants in tomato cultivation, reducing the impacts of these practices on the

Table 2. Evaluation of growth promotion in purple passion fruit seedling, and biological control of F. oxysporum. Means with the same letter are not significantly different (LSD Fisher p > 0.05) for (n=3).

Strain	Phosphorus solubilization		IAA (µg/mL)		Antibiosis (%)		VOC (%)	
UNE004	-0.14	F	4.54	BC	63.89	EF	3.79	C
UNE010	-0.8	BC	4.81	A	27.47	B	1.27	C
UNE017	0.91	AB	11.99	D	37.5	C	10.56	B
UNE022	-0.42	DE	7.59	C	20.95	A	1.25	C
UNE028	-0.13	F	3.45	AB	52.24	D	12.46	B
UNE063	-0.57	CD	0.38	A	52.16	DE	1.89	C
UNE067	-0.2	EF	2.14	AB	58.99	D	3.17	C
UNE075	-0.07	F	1.18	AB	67.63	F	33.78	A
UNE098	-1.11	A	0.22	A	54.96	D	32.32	A

environment and providing a more sustainable agriculture (Corrales et al., 2014).

It was found that *Fusarium equiseti* UNE017 and *Trichoderma asperellum* UNE098 had absorbances of -0.91 and -1.11, respectively, indicating that they are efficient solubilizing strains. Mehta and Nautiyal (2001) reported that the most efficient solubilizers achieve a decolorization limit of -1.99 of the NBRI-bromophenol blue medium. UNE017 and UNE098 are endophytes of interest to study the effect of their inoculation on plants whose substrate presents phosphorus limitations.

Inoculation with UNE067, identified as *Curvularia penniseti* by Hurtado (2020) and Table 1, slightly increased the leaf area of the *P. edulis* f. *edulis* plants. The increase in leaf area was closely related to the increase in fresh and dry weight since leaves are a light-harvesting organ, acting as a source organ by translocating carbohydrates to sink organs such as stems, roots and new leaves (Barrientos-Llanos et al., 2015). Growth promotion is an ability that has been found in several genera of endophytic fungi (Hossain et al., 2017; Şesan et al., 2020; Silva et al., 2006; Zapata et al., 2019).

The genera *Trichoderma*, *Alternaria*, and *Fusarium* have been reported in Passifloras endophytes such as *P. caeruela* and *P. edulis* f. *flavicarpa*, which increase leaf size and biomass (Şesan et al., 2020; Silva et al., 2006). In addition, endophytic strains of the *Curvularia* genus have been identified as plant growth promoters since their inoculation increased leaf area and number of leaves in several plants, such as pigeon peas and *P. caerulea* (Priyadharsini and Muthukumar, 2017; Şesan et al., 2020).

The purple passion fruit material grown in Colombia has high genetic similarity; however, broad variability has been found in the expression of phenotypic characteristics (morphoagronomic and ecophysiological) because of its plasticity, domestication processes and the phenomenon of self-incompatibility (Rodríguez, 2019). This phenotypic variability could lead to biases in the interpretation of data in an *in vivo* assay although these are offset by the number of biological replicates.

The interactions between microbes that are observed at the *in vitro* level can show how the behavior of a fungus of interest will be in a natural environment, hence the importance of evaluating them (Badalyan et al., 2004). In the case of the dual culture interaction, biocontrol can be largely attributed to physical contact between microbes (Li et al., 2018).

In the "type C: replacement", interactions observed for the endophytes UNE017, UNE063, UNE004, UNE075, and UNE098 had close physical contact that allowed overgrowth, colonization, and mechanisms of action, such as hyperparasitism (Sánchez-Fernández et al., 2013).

UNE004, UNE075 and UNE098 belong to the genus *Trichoderma* (Table 1) and have a strong antagonistic activity with PRGI, 63.89 %, 67.63 % and 54.96 %, respectively. Similar results were reported by Dos Santos et al. (2011), who found that isolates of foliar endophytes from passion fruit, a species of the same family as purple passion fruit, generate growth inhibition of the pathogen *Fusarium* sp. with averages close to 60 %. Duarte-Leal et al. (2018) found that *T. asperellum* strains inhibited the growth of two pathogenic bean strains: *F. dlaminii* and *F. solani*, between 41 % and 48.72 %, respectively.

Under *in vitro* conditions, biocontrol can also be attributed to the production of various molecules that are transported by water in a culture medium, known as diffusible metabolites (Duarte-Leal et al., 2018; Li et al., 2018). This is found in the "B" interaction type, Inhibition at a distance, which was observed for the UNE010 and UNE022 strains.

Epiccoccum nigrum UNE022 (table 1) is a biological control agent that produces diverse compounds, including antifungals, flavin, melin and epicoccolide A, B, which have been described in studies on the fungus *Botrytis cinerea* (Elkhateeb and Daba, 2019). Effects have also been observed on fungi of the *Fusarium* genus, which affect sugarcane cultivation, inhibiting the *in vitro* growth of *F. verticillioides* by more than 50 %. In the present study, a zone of inhibition formed between the strains, which was evidence of the excretion of diffusible compounds into the medium (Fávaro et al., 2012).

Table 3. Fresh and dry weight of leaves, stems, roots, and whole plants of purple passion fruit (*P. edulis* Sims f. *edulis*) at 63 days after inoculation with the respective endophytic fungi. Means with the same letter are not significantly different (LSD Fisher p > 0.05), for six replicates.

Strain	Fresh weight (g)								Dry weight (g)							
	Leaves		Stem		Root		Total		Leaves		Stem		Root		Total	
Control	6.21	AB	3.10	ABC	4.17	AB	13.89	AB	1.30	A	0.57	AB	0.40	AB	2.28	AB
UNE004	5.65	AB	3.04	ABC	3.51	B	12.2	ABC	1.17	A	0.67	AB	0.39	AB	2.15	AB
UNE010	5.20	BC	2.57	BC	3.24	B	11.00	BC	1.06	AB	0.46	AB	0.34	AB	1.85	AB
UNE017	5.77	AB	2.87	BC	4.19	AB	12.82	ABC	1.21	A	0.55	AB	0.39	AB	2.17	AB
UNE022	6.43	AB	3.56	ABC	3.91	AB	13.89	AB	1.41	A	0.67	AB	0.46	AB	2.53	AB
UNE028	2.28	C	2.02	C	3.23	B	6.59	C	0.5	B	0.39	B	0.27	B	1.38	B
UNE063	4.75	BC	2.17	BC	3.39	B	10.83	BC	1.04	AB	0.51	AB	0.31	AB	1.88	AB
UNE067	8.41	A	4.62	A	6.07	A	18.05	A	1.66	A	0.79	A	0.46	AB	2.98	A
UNE075	6.01	AB	3.87	AB	4.62	AB	14.50	AB	1.32	A	0.67	AB	0.47	A	2.46	AB
UNE098	4.94	BC	2.62	BC	4.1	AB	11.65	BC	1.05	AB	0.50	AB	0.39	AB	1.92	AB

The "type A" interaction is the result of competition for limited resources and is a natural phenomenon that regulates the distribution and abundance of fungi in the ecosystem. *Trichoderma* is cosmopolitan and dominates many different niches, making it stand out among biological controllers since it allows adaption to different ecological conditions (Badalyan et al., 2004). Although most responses are attributed to cell-cell contact, volatile compounds also play an important role in the biocontroller-pathogen interaction. Fungi of the genus *Trichoderma* increase the production of antifungals, including VOCs in response to the presence of *F. oxysporum* by recognizing the VOCs produced by the pathogen (Li et al., 2018).

The results showed that two strains of *T. asperellum*, UNE075 and UNE098 (identification in Table 1, and Hurtado, 2020), reduced the radial growth of *F. oxysporum*, close to 30 % because of the action of VOC, probably because species in the *Trichodema* genus produce volatile antifungal compounds, such as 3-octanono, 1-octen-3-ol, 2-ethyl-1-hexanol, 1-nonanol, and 6-pentyl-2H-pyran-2-one (Li et al., 2018; Wonglom et al., 2020). The VOCs produced by *T. asperellum* not only have a direct role in controlling pathogens but also have effects on plant growth and the induction of defense responses (Wonglom et al., 2020).

It is possible that, to achieve important impacts in the management of purple passion fruit crops, the possibility of testing different co-inoculations with endophytes with more potential (UNE004, UNE098 and UNE075) should be explored since they could achieve complementary effects and greater effectiveness than with bio-controllers used individually (Arango et al., 2012).

CONCLUSIONS

Our results suggest that fungal endophytes could be capable of mitigating the effect caused by *F. oxysporum* in purple passion fruit plants, due to their *in vitro* behavior, in which six of the strains evaluated showed a high degree of inhibition of major mycelial growth 50%, through direct interaction in dual culture. *T. asperellum* (UNE075 and UNE098) produces volatile organic compounds that alone inhibit the growth of the pathogen by more than 30%. These characteristics are desirable in the formulation of bioproducts for the management of the disease and in improving the health of the soils.

It was found that *T. asperellum* (UNE098) and *F. equiseti* (UNE017) could play a role in the bioavailability of minerals such as phosphorus. For their part, *E. nigrum* (UN022) and *F. equiseti* (UN017) present IAA production, recognized as attributes related to the promotion of plant growth (PPG).

To evaluate the PPG effect *in vivo*, a test was carried out on purple passion fruit seedlings which were inoculated with the endophytic strains. *C. penniseti* UNE067 generated a slight increase in biomass. The results of this work reveal that the strains of symbiotic microbes evaluated could have different useful biotechnological applications in the development of sustainable production models.

AUTHOR'S PARTICIPATION

Stephany –HURTADO CLOPATOSKY

ROLES: investigation, visualization, methodology, interpretation of results, formal analysis, writing – original draft, writing – review & editing.

Luz Marina –MELGAREJO MUÑOZ

ROLES: Conceptualization, Methodology, Supervision, Writing – review & editing. Funding acquisition

Ginna Natalia CRUZ - CASTIBLANCO

ROLES: Data curation, statistical data analysis, Writing – review & editing.

Lilliana María–HOYOS CARVAJAL

ROLES: Conceptualization, Methodology, Supervision, Writing – review & editing.

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CONFLICT OF INTEREST

The authors of this manuscript indicate that they have no conflict of interest.

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