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OVERVIEW OF FUNGAL SPECIES ASSOCIATED TO YELLOW
PITAHAYA PLANTATIONS, *Selenicereus megalanthus*, IN THE
ECUADORIAN AMAZON REGION

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DEDICATORIA

A los maestros que me formaron durante toda mi vida estudiantil y aportaron con sus conocimientos y consejos para completar con éxito esta etapa de mi vida.

A mis padres y hermanos, quienes sentaron en mi las bases de la responsabilidad y el deseo de superarme.

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RESUMEN

La producción de pitahaya amarilla *Selenicereus megalanthus* (K. Schum. Ex Vaupel) Moran, para exportación representa una alternativa para los agricultores de varias provincias de la Amazonía ecuatoriana. A medida que aumenta la demanda en los mercados internacionales y el área de cultivo crece, se requieren nuevas tecnologías agrícolas. En Ecuador, para cultivos de pitahaya, no se dispone de información sobre hongos endófitos. En consecuencia, este estudio tuvo como objetivo principal identificar especies de hongos endófitos en tres cantones: Tena, Santa Clara y Palora. Se aislaron un total de 65 colonias en agar de dextrosa de papa (PDA) y se conservaron en glicerol al 75%. La selección de muestras para la identificación molecular se basó en características morfológicas únicas, esto ayudó a evitar duplicados. Se secuenció la región genómica ITS, las secuencias se ensamblaron con MEGA11 y se alinearon en el NCBI BLAST. El análisis filogenético permitió la asignación de 26 cepas a tres órdenes: Xylales (16), Diaporthales (6) e Hypocreales (4). En cuanto al tipo de tejido vegetal, 13 cepas están asociadas a tejidos sanos y 14 a tejidos enfermos, donde *Fusarium foetens* se encuentra en los dos tipos de tejidos. Estos hallazgos son el punto de partida para comprender las asociaciones de hongos endófitos con los cultivos de pitahaya amarilla. Las cepas aisladas, identificadas y preservadas podrían utilizarse en futuras investigaciones, pues un mejor conocimiento de la interacción planta-hongo y los metabolitos beneficiosos podría contribuir en el planteamiento de mejores prácticas agrícolas.

Keywords: Hongos endófitos, pitahaya amarilla, identificación molecular, filogenia ITS, Amazonia ecuatoriana

ABSTRACT

Production of yellow pitahaya *Selenicereus megalanthus* (K. Schum. Ex Vaupel) Moran, for exportation, represents an alternative for farmers in several provinces of the Ecuadorian Amazonia. As a result of demand increase in the international markets, plantation areas also increase. In Ecuador, for pitahaya crops, to our knowledge, no information is available on endophytic associations. Therefore, this study aimed to identify endophytic fungi species in three cantons: Tena, Santa Clara, and Palora. 65 colonies were isolated in potato dextrose agar (PDA) and preserved in 75% glycerol. Unique morphological features were used to select samples for molecular identification without duplicates. The Internal Transcribed Spacer (ITS) region was sequenced, sequences were assembled using MEGA11, and aligned with the NCBI BLAST. Phylogenetic analysis allowed the assignment of 26 strains to three orders Xylales (16), Diaporthales (6), and Hypocreales (4). Regarding tissue type, 13 strains were associated with healthy and 14 with diseased plant tissues, *Fusarium foetens* was found in both types of plant. These findings start our understanding of endophytic fungi related to yellow pitahaya crops. And the isolated, identified and preserved samples could be used for future research since better knowledge of plant-fungi interaction and beneficial metabolites could contribute to the proposal of better agriculture practices.

Keywords: Endophytic fungi, Yellow pitahaya, Molecular identification, ITS phylogeny, Ecuadorian Amazonia

1. INTRODUCTION

Microscopic fungi are organisms that play essential roles in nature participating in diverse processes, including organic matter recycling, soil formation and conservation, and balancing ecosystems due to their ability to interact with other organisms (Deacon, 1993; Hawksworth, 1997). A type of relationship with growing significance in agricultural research is the mutualistic associations between microscopic fungi and plants. Several plant species of economic importance interact with species of organisms characterized as endophytes (Proença et al., 2017).

De Bary (1866), defined endophytes for the very first time as microorganisms that interact with plant tissues. Currently, it is defined as microorganisms that colonize plant tissue asymptotically (Sánchez et al., 2013; Yan et al., 2019) and do not damage plant tissue, rather there may be a mutual benefit since the survival of both the plant and the fungus is increased (Hyde and Soyong, 2008; Bacon and White, 2016). In general, plants host the fungus and provide food and protection. On the other hand, endophytes confer adaptive potential to the plants against adverse conditions (Abello and Kelemu, 2006). The most important outcome is an increase in plant resistance to biotic and abiotic stress (Busby et al., 2016)

Several studies show that endophytic fungi protect and help their hosts to resist biotic stress caused by phytopathogens and herbivores (Bourassa, Brodeur and Carrière, 2007; Paz et al., 2007; Rodrigo et al., 2017; Yao et al., 2017). Endophytes are involved in the production of antimicrobial metabolites (Mousa et al., 2015; Rodrigo et al., 2017) when competing for nutrients and space along with pathogenic microorganisms (Ownley et al., 2008; Siddaiah et al., 2017), additionally, they can intervene within the immune response of plants, inducing the production of other toxic substances harmful to herbivores (Yan et al., 2019), the endophytic fungi that possess this particular ability to protect plants from herbivores are known as entomopathogenic fungi.

Considering the loss of crops due to abiotic stress, Khan *et al.* (2013), indicates that endophytic fungi also have the ability to promote tolerance to adverse environmental factors in their hosts. These include the increased tolerance to drought (Zhang and Nan, 2007), excess salts or acid soils (Waller et al., 2005), and soils contaminated with heavy metals (Fässler et al., 2010). Moreover, endophytes can act as plant growth regulators or promoters by producing plant hormones (Hiruma et al., 2016; Yan et al., 2019).

Nevertheless, endophytic fungi interacting with symptomatic plants may also be cataloged as pathogenic endophytes or latent pathogens, causing damage when the host is exposed to stress conditions (Mishra et al., 2021).

Therefore, identification of endophytic fungi is crucial since biological control mediated by native endophytes is a determinant of plant health (Munir et al., 2020), and favors yield. In addition it does not alter the balance of ecosystems (Kalimutu et al., 2020). Some traditional methods such as morphological identification are based on the analysis of macroscopic and microscopic structures, including mycelial staining or description of *in vitro* culture (Shenoy et al, 2007; Piontelli, 2011), however, although traditional techniques allow microscopic identification of fungi, it is necessary to validate and complement the obtained information with more up-to-date methods, i.e. molecular biology techniques, where DNA sequencing plays the major role. These techniques make research of the phylogenetic relationship between fungal species and fungal-plant interaction easier and more precise (Abello and Kelemu, 2006).

Yellow pitahaya or *Selenicereus megalanthus* belongs to Cactaceae family. The plant is native to the tropics of America and widely distributed within Mexico, Venezuela, Central America, Antilles, Bolivia, Peru, Ecuador, and Colombia due to its high adaptation capacity (Kondo et al., 2013; Sotomayor et al., 2019). **Figure No. 3** (methods section) shows yellow pitahaya plant with concave or smooth stems, also known as cladodes, hermaphroditic flowers of about 25 cm long, yellow oval-shaped fruits weighing between 50 to 400 g, and sweet pulp with dark-colored seeds (Creucí, 2015). Fruits are mainly cultivated for their appearance and nutraceutical properties (Suárez, 2011). In Ecuador, two ecotypes are produced: Pichincha or Nacional ecotype with fruits up to 150 g, cultivated in the northwest of Pichincha province since 2000; and a few years later in Morona Santiago, arising the Palora ecotype (Molina et al., 2009; Trujillo, 2014). According to Vargas *et al.* (2020), a total of 7,498.80 Tm were exported in 2019 representing more than 44 million dollars of income for Ecuador.

In Ecuador, microscopic fungi identification is predominantly linked to crops of great economic importance for the country, namely, bananas, palm oil, cocoa, among others (Thomas et al., 2008; Avilés and Granja, 2014; Martínez et al., 2016; Abedrabbo, 2017; Terrero et al., 2017). However, yellow pitahaya crops have no reports on endophytic fungi, despite being a crop highly accepted in the European and United States markets

in recent years (Vilaplana et al., 2018), with an economically promising future for farmers (Kondo et al., 2013), especially, in the Ecuadorian Amazonia region where this alternative crop has helped to reduce the unemployment rate and to raise the living standard of the farmers (Erazo and Parra, 2013).

In addition, the lack of information limits investigations for the design and use of new ecological alternatives that benefit the crop and may contribute to the mitigation of negative effects caused by pathogenic organisms (Kumar et al., 2019), responsible for causing significant economic losses in agriculture (Yan et al., 2019). Endophytic fungi can represent a promising solution as biocontrol agents and as a source for biofertilizers formulation, so the objective of this research was to isolate, molecularly identify, and describe the species of endophytic fungi associated with plantations of yellow pitahaya, *Selenicereus megalanthus*, in the Ecuadorian Amazon region. Thus providing novel information for future research.

1.1. METHODS

1.2. Sampling

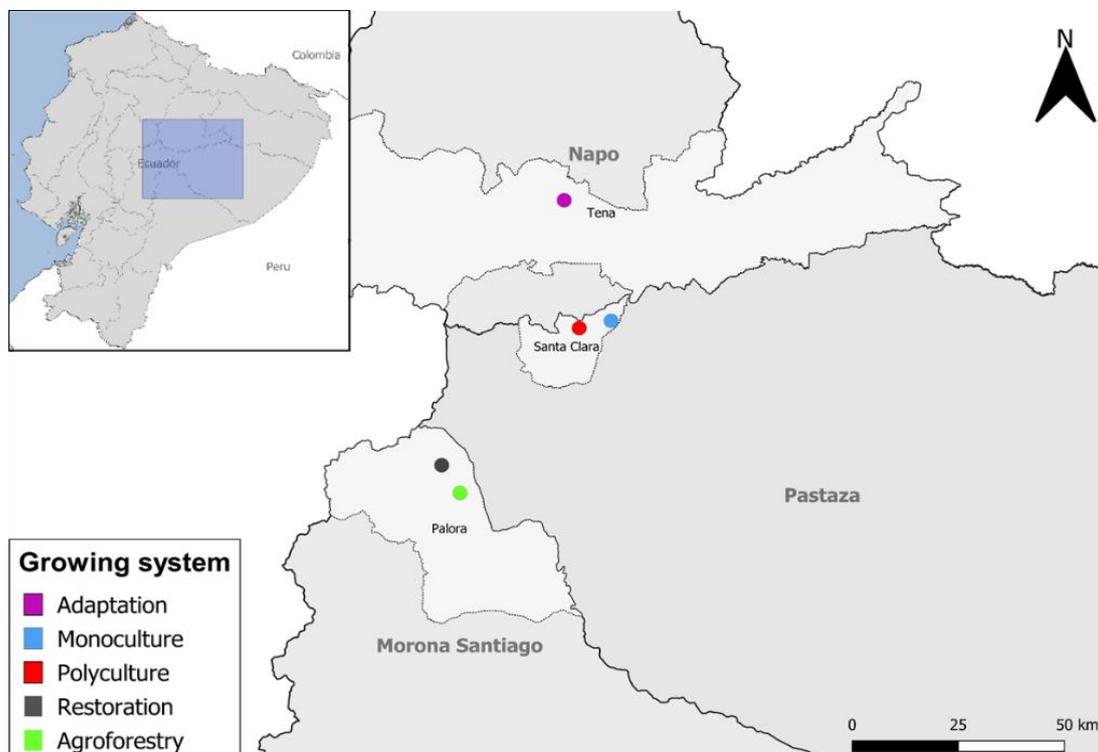


Figure 1: Geographical location map of sampling areas according to the growing systems.

Colored dots represent growing systems. **Made by:** Rocano, 2022.

Plantations from three Amazonian provinces located in three cantons were selected. Samples were taken of 5 different types of agrosystems: monoculture, polyculture, agroforestry, a plantation that was under restoration in Palora, and a plantation in adaptation phase in Tena. (**Figure No. 1**). The agroforestry system is characterized by pitahaya growing along with legumes plants: *Eryhrina poeppigiana*, *Gliricidia sepium*, and *Flemingia macrophylla*. The polyculture system, has a mixed crop production together with *Carica papaya L.*(papaya), *Inga edulis* (guaba), *Colocasia esculenta* (chinese potato), *Cymbopogon citratus* (hierba Luisa) and *Vanilla planifolia* (vainilla). The monoculture systems had exclusively pitahaya plants. The restoring system is a plantation untreated with fertilizers or fuming for approximately three years. The adaptation system is a plantation in adaptation phase, as yellow pitahaya is a relatively new crop at Napo province. Plants were identified as symptomatic if any cladode or fruit had a diseased tissue, while diseased-free plants were classified as asymptomatic (**Figure No. 2**). Sampling was performed at random locations in each plantation, and selected cladodes and fruit tissues were cut in sections (**Figure No. 3**), kept in a pre-labeled plastic bag, and transported in an ice cooler to the laboratory.

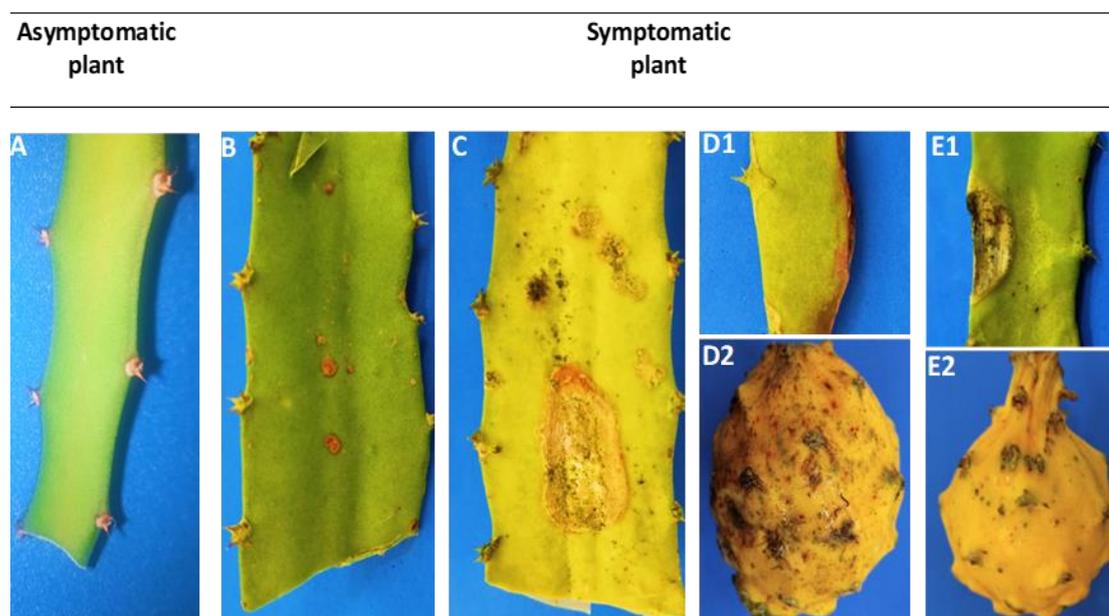


Figure 2: Samples of types of tissues. (A). Cladode of asymptomatic plant. (B), (C), (D1), (E1), (D2), (E2). Cladodes and fruits of symptomatic plant. **Made by:** Rocano, 2022

Disinfection of the external surface of cladodes and fruits was carried out by treating the tissues with 2% and 5% chlorine solution, including rinsing steps with sterile distilled

1.4. DNA extraction, amplification and purification

Approximately, 100 mg of mycelium was collected in a 1.5 ml tube, frozen on liquid nitrogen and macerated till dust consistency. Genomic material extraction was accomplished by following the procedure of the E.Z.N.A. Fungal DNA Kit and extracted DNA was used as a template for the polymerase chain reaction (PCR) of the Internal Transcribed Spacers (ITS), a component of the eukaryotic 40s ribosomal subunit widely used for the study of microscopic fungi (Romo, 2014). A Phusion DNA polymerase and universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'TCCTCCGCTTATTGATATGC-3') described by White *et al.* (1990) were used for the amplification, the PCR program seattle was: 98 °C for 10 seconds, 35X cycles: denaturation 98 °C during 30 s; alignment 55 °C during 30 s; extension at 72 °C during 30 s; elongation at 72 °C for 10 minutes. Amplification efficiency was tested by electrophoresis in a 1% agarose gel. Amplicons were purified with the Wizard Genomic DNA Purification Kit, and sequencing was carried out following the Sanger methodology with the application of the BigDye Terminator v3.1 Cycle Sequencing Kit, slightly modified, on an ABI 3130 sequencer.

1.5. Bioinformatic analysis

Bioinformatic analysis of sequences was accomplished on MEGA11 software (Tamura *et al.*, 2021), and alignments for consensus sequences were performed using the clustalW algorithm. Obtained consensus sequences were analyzed with the basic local alignment search tool (BLAST), and sequences with the highest similarity were used for assigning genus or species identification to each isolated strain (Necochea and Canul, 2004). Phylogenetic tree was assembled employing the Bootstrap method (1000 repetitions), with sequences obtained from GenBank and sequences of molecular identification. Thus, two phylogenetic trees were constructed, one for fungi isolated from asymptomatic plants and one for fungi isolated from symptomatic plants. In both cases, the fungal species *Alternaria alternata*, which belongs to the Pleosporaceae family (Ahmmed *et al.*, 2020) was chosen as an out-group.

2. RESULTS

2.1. General morphological description

Each colony's macroscopic characteristics and microscopic characteristics were reported as shown in **Figure No. 4**. Macroscopic characteristics of isolates from different culture systems: colony elevation, surface, texture, and frontal and reverse pigmentation (**Figure No. 4a, 4b**), were reported. Isolated colonies exhibited diverse colors, ranging from white to black (including orange, gray, and pink); as well as varied textures, elevations, and surfaces. General microscopic characteristics of each fungus were also documented (**Figure No. 4c, 4d, 4e**). The majority of colonies had septated hyphae, while reproductive structures often do not develop early but some sexual reproductive structures such as oogonium and asexual spores were observed, being the most common phragmospores. In some cases, reproductive structures were not observed even after 20 days of culturing. A detailed summary of the general macroscopic and microscopic characteristics is presented in **Annex No. 1**.



Figure 4: Characteristics of isolated colonies of *Pseudopestalotiopsis* genus in PDA medium. (a), (b) Macro of fungi on a petri dish. (c), (d), (e) Micro of fungi.

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2.2. Molecular identification

Using molecular tools and literature review was possible to identify a total of 26 species of fungi associated with yellow pitahaya (**Table No. 1**). The 9 genera identified are distributed in the two types of plants, symptomatic and asymptomatic, from the 5 different plantations (**Figure No. 5**). *Nigrospora* genus was only observed in the plantation that was in adaptation phase; *Pseudopestalotiopsis* genus in adaptation phase and polyculture plantations; *Fusarium* genus was identified in three types of plantations, adaptation, restoration, and polyculture; *Acremonium* genus was identified in the agroforestry growing system; *Neopestalotiopsis* genus was identified in polycultures and monocultures, while *Astrocystis*, *Cordyceps*, and *Nemania* genus were identified only in monocultures.

From 65 isolated cultures, colonies with equal morphological characteristics were not considered for genetic identification. Out of 38 strains molecularly identified; 31 are reported in this study, according to the literature review 27 are classified as endophytes (4 duplicates), 3 are latent saprophytic as endophytes (1 duplicate), and 1 is entomopathogenic as an endophyte in this plant. 7 are not reported as they do not present endophytic associations, they only have been reported as pathogens in other plants. Considering percentages: 71% are endophytes, 8% are saprophytes, 2% are entomopathogenic and 19% do not present endophytic association with yellow pitahaya plants. Overall 26 strains, without considering duplicates and those that do not present endophytic associations are presented in **Table No. 1**.

Table 1: Species information and Genbank accession number of sequences.

Orden	Genus	Family	Accession Number	Collection spot
Diaporthales	Diaporthe	<i>Diaporthe endophytica</i>	NR_111847.1	Palora, Morona Santiago
		<i>Diaporthe maytenicola</i>	NR_137826.1	Santa Clara, Pastaza
		<i>Diaporthe miriciae</i>	NR_147535.1	Palora, Morona Santiago
		<i>Diaporthe novem</i>	NR_111855.1	Santa Clara, Pastaza
		<i>Diaporthe passifloricola</i>	NR_147595.1	Santa Clara, Pastaza
		<i>Diaporthe terebinthifolii</i>	NR_111862.1	Palora, Morona Santiago
Hypocreales	Acremonium	<i>Acremonium sclerotigenum</i>	NR_149332.1	Palora, Morona Santiago
	Cordyceps	<i>Cordyceps javanica</i>	NR_111172.1	Santa Clara, Pastaza
	Fusarium	<i>Fusarium boothii</i>	NR_121203.1	Palora, Morona Santiago

		<i>Fusarium foetens</i>	NR_159865.1	Tena, Napo
	Astrocystis	<i>Astrocystis bambusicola</i>	NR_158350.1	Santa Clara, Pastaza
	Nemania	<i>Nemania abortiva</i>	NR_121350.1	Santa Clara, Pastaza
Xylariales	Nigrospora	<i>Nigrospora aurantiaca</i>	NR_153477.1	Tena, Napo
		<i>Nigrospora camelliae-sinensis</i>	NR_153473.1	Tena, Napo
		<i>Nigrospora hainanensis</i>	NR_153480.1	Tena, Napo
		<i>Nigrospora lacticolonia</i>	NR_153471.1	Tena, Napo
		<i>Nigrospora oryzae</i>	KF192823.1	Tena, Napo
		<i>Nigrospora osmanthi</i>	NR_153474.1	Tena, Napo
		<i>Nigrospora pyriformis</i>	NR_153469.1	Tena, Napo
		<i>Nigrospora rubi</i>	NR_153470.1	Tena, Napo
		<i>Nigrospora vesicularifera</i>	NR_165927.1	Tena, Napo
		<i>Nigrospora vesicularis</i>	NR_153479.1	Tena, Napo
Pseudopestalotiopsis	<i>Pseudopestalotiopsis cocos</i>	NR_145246.1	Tena, Napo	
	<i>Pseudopestalotiopsis theae</i>	NR_111716.1	Tena, Napo	
	<i>Pseudopestalotiopsis solicola</i>	NR_161086.1	Tena, Napo	
Neopestalotipsis	<i>Neopestalotipsis formicarum</i>	NR_145242.1	Santa Clara, Pastaza	

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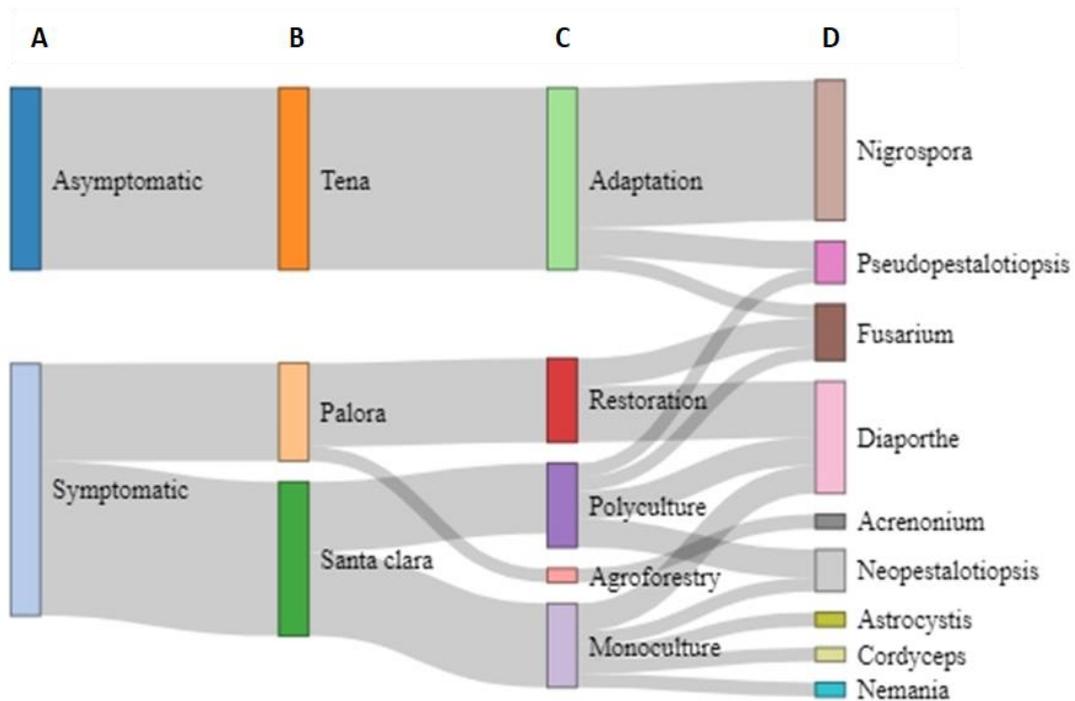


Figure 5: Relationship between plantations and identified fungi genera: (A) Type of the plant. (B) Location. (C) Plantations. (D) Genera of fungi. **Made by:** Rocano, 2022

As a whole, a high degree of congruence is shown within the phylogenies at order level (Figure No. 6A, 6B). In the phylogenetic tree formed by the sequences of identified

fungi from symptomatic plants (**Figure No. 6A**), all phylogenies are divided into three branches: Xylales, Hypocreales, and Diaporthales. The Xylales order formed a clade with 91% of Bootstrap support (BP) with four genera of fungi *Pseudopestalotiopsis*, *Neopestalotiopsis* and *Nemania*. The order Hypocreales formed a clade with 67% BP, with three genera of fungi *Cordyceps*, *Acremonium*, and *Fusarium*. And, in the last order, the Diaporthales formed a well-supported clade with 90% BP, with 6 species on the *Diaporthe* genus.

Concerning asymptomatic plants, within the phylogenetic tree (**Figure No. 6B**), two clades are formed with Hypocreales order with 100% BP, where there is a single clade species *Fusarium foetens*, meanwhile, clade formed for the order Xylales with 100% BP presents two clades of genera; *Pseudopestalotiopsis* genera with two species *Pseudopestalotiopsis cocos* and *Pseudopestalotiopsis solicola* and *Nigrospora* genus with 10 identified species *Nigrospora rubi*, *Nigrospora hainanensis*, *Nigrospora vesicularifera*, *Nigrospora camelliae-sinensis*, *Nigrospora pyriformis*, *Nigrospora aurantiaca*, *Nigrospora oryzae*, *Nigrospora osmanthi*, *Nigrospora lacticolonial* and *Nigrospora versicularis*.

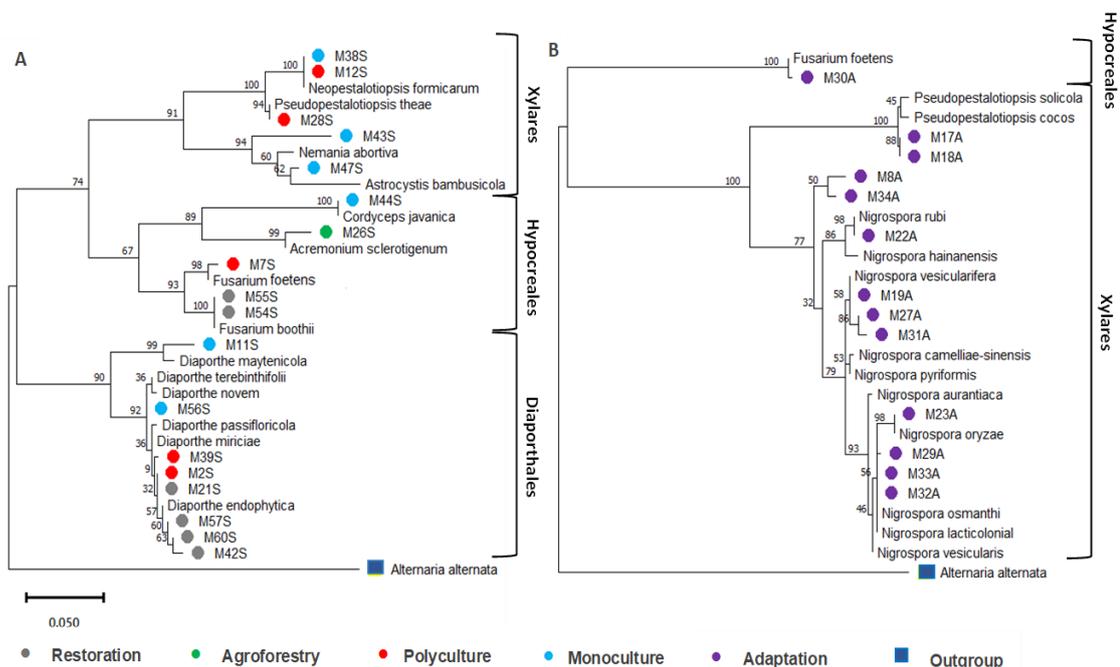


Figure 6: Maximum likelihood phylogeny for 40S ribosomal subunit. (A) Symptomatic plants. (B) Asymptomatic plants. Colored circles represent the growing systems. Made by: Rocano, 2022

3. DISCUSSION

Genetically identified strains underwent an exhaustive literature review before categorizing them as endophytic fungi. Thus, isolated fungi from asymptomatic tissues of yellow pitaya were cataloged as endophytes as they were present on non-diseased plants, results were supported by literature while fungi isolated from symptomatic tissues were classified as endophytes according to a literature review (Sessa et al., 2018; Belfiori et al., 2021). Some authors have reported the same fungi species as endophytes, pathogens, or saprophytes in other plant hosts, hence, it is feasible to discuss whether they are endophytes, pathogens, latent saprophytes, or could belong to endophytic latent pathogens (Sessa et al., 2018; Karolina et al., 2019; Belfiori et al., 2021).

Fungi of the *Nigrospora* genus isolated from asymptomatic pitahaya plants are widespread in numerous plant hosts (Wang et al., 2017). Isolated species: *N. rubi*, *N. hainanensis*, *N. vesicularifera*, *N. Camelliae-sinensis*, *N. pyriformis*, *N. aurantiaca*, *N. oryzae*, *N. osmanthi*, *N. lacticolonia* and *N. versicularis*, were also reported as endophytes by Wang et al. (2017), in different plants like *Camelliae sinensis*, *Castanopsis* sp., *Musa paradisiaca*, *Rhododendron* sp., *Oryza sativa*, *Osmanthus* sp., *Rosa* sp., and *Rubus* sp.

Individuals from genera *Neopseudopezalotiopsis* and *Pseudopezalotiopsis* are reported to cause disease in various hosts (Gualberto et al., 2021), in this work, *P. cocos* and *P. solicola* were isolated as endophytes, however, the type of relationship that shares with pitahaya plants is still unclear. Compant et al. (2016) indicate that despite being organisms isolated from asymptomatic plants these may be either saprophytic or latent pathogens. *P. theae* species isolated from asymptomatic plants have been identified as an endophytic fungus in *D. aphyllum* (Sopalun and lamtham, 2020).

In the case of strains isolated from symptomatic plants, Gomes et al. (2013), mention that fungi of the genus *Diaporthe* have a wide variety of hosts and can be classified as endophytes, pathogens, or saprophytes. Belonging to endophytes *D. endophytica* and, *D. terebinthifolii* in *Maytenus ilicifolia* and *Schinus terebinthifolius*, respectively, while,

Ferreira *et al.* (2017) reported the endophyte *D. maytenicola* in *Vellozia gigantea*. In other cases, the same specie has been reported as endophyte or pathogen, such as the case of *D. novem* identified as an endophyte (Gomes *et al.*, 2013), and as a pathogen in diseased soybeans tissues (Santos *et al.*, 2011); *D. passifloricola* reported as an endophyte in *Citrus grandis* (Dong *et al.*, 2021), and cataloged as causal agent of the stem-end rot disease in *Citrus reticulata* (Chaisiri *et al.*, 2021).

Regarding the genus *Fusarium*, the species belonging to this genus are mainly considered pathogenic organisms, such as the case of *F. boothii* isolated from barley tissues with head blight symptoms (Cerón-Bustamante *et al.*, 2018), meanwhile, in soybean *Glycine max* (L.) Merr was categorized as an endophyte fungus (Batzer and Mueller, 2020); on the other hand, *F. foetens* was identified as a pathogen in *Begonia elatior*, causing tracheomycosis (Schroers *et al.*, 2004), and recognized as an endophyte in tomato stem (Imazaki and Kadota, 2015).

Other underrepresented genera identified in symptomatic plants have also been reported as endophytes, this is the case of *Acremonium sclerotigenum* isolated from *Terminalia bellirica* (Prathyusha *et al.*, 2015), and *Nemania abortiva* isolated from *Vellozia gigantea* (Prathyusha *et al.*, 2015). Additionally, the last two molecularly identified species that have been reported as saprophytes, *Astrocystis bambusicola* (Wu *et al.*, 2021) and *Neopestalotiopsis formicarum* (Maharachchikumbura *et al.*, 2014), could be associated with endophytic latent saprophytes, as indicated by Promputtha *et al.* (2007), some endophytic fungi change their type of interaction and become saprophytic when the host plant is in the senescence stage.

Even Though *Cordyceps javanica* species was isolated from diseased tissue, there is a lack of information on interaction with plants. This strain was identified as an entomopathogen by Ou *et al.* (2019), in the cadaver of *Diaphorina citri*, one of the most serious citrus insect pests in the world.

Our analysis suggests that identified fungi present associations with asymptomatic and symptomatic tissues of yellow pitahaya plants in different types of growing systems. However, despite we have carried out an exhaustive analysis of literature to identify possible interaction of fungi with plants, it is necessary to complement it with other experimental tests such as pathogenicity, antagonism tests, among others (de Almeida

et al., 2020; Kalimutu et al., 2020; Tembo et al., 2020), to verify if the identified fungi are endophyte, natural pathogens, saprophytes or pathogen in the latent phase of its life cycle.

4. CONCLUSION

Yellow pitahaya plants were examined for the first time to identify endophytic fungi in asymptomatic and symptomatic tissues, providing novel information about fungal endophytic communities associated with fruits and cladodes of plants grown in some of the productive areas of the Ecuadorian Amazonia, highlighting the necessity of further research. Furthermore, preserved strains could be used for future tests on biological control of fungal pathogens of yellow pitahaya, as well as for screening useful bioactive molecules.

From all collected samples, 26 species of endophytic fungi associated with asymptomatic and symptomatic yellow pitahaya plants grown in the productive area of the Ecuadorian Amazon region were identified by the molecular analysis of ribosomal ITS which gives reliable and relatively faster results compared to traditional identification techniques. The dominant species identified molecularly in asymptomatic plants belong to the genus *Nigrospora*, while in symptomatic plants they belong to the genus *Diaporthe*. According to a literature review, regarding plant species, and host living conditions some of these species of fungus may have more than one type of interaction with the host, either true endophyte, latent pathogenic, or saprophytic. A set of 18 identified species of fungi may be correlated to potential pathogens or saprophytes, remaining in the latent phase of their life cycle, hence, this species could belong to the pathogen endophyte classification. Therefore further investigations have to be carried out to have a better understanding of the beneficial or detrimental relation to the host, in this case, to yellow pitahaya plants.

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Annexes

Annex 1. Isolated fungal strains for each type of growing system.

Farm type	Code	Form	Elevation	Surface	Texture	Front pigmentation	Reverse pigmentation	Somatic structure	Reproductive structure
Agroforestry	M265	Circular	Flat and extended	Plicate	Cottony	White with gray	Light brown	Septate	Merosporangio
Agroforestry	M455	Circular	Flat and extended	Cerebriform	Cottony	Beige with white inner halo	Light brown	Septate	Not observed
Agroforestry	M485	Irregular	Flat and extended	Plicate	Cottony	Gray	White	Septate	Not observed
Agroforestry	M515	Circular	Flat and extended	Sectorized	Cottony	White w. black spots	Cream	Septate	Oogonio
Agroforestry	M595	Rhizoidal	Flat and extended	Radial grooves	Velvety	White	Cream	Haustorio	Not observed
Asymptomatic	M17A	Irregular	Umbilicated convex	Sectorized	Pulverulent	Light orange	Light orange	Septate	Not observed
Asymptomatic	M18A	Irregular	Umbilicated convex	Sectorized	Pulverulent	Light orange	Light orange	Septate	Not observed
Asymptomatic	M19A	Circular	Elevated and limited	Sectorized	Velvety	White	Light brown	Septate	Not observed
Asymptomatic	M22A	Circular	Flat and extended	Plicate	Velvety	White w. orange center	White with orange center	Septate	Not observed
Asymptomatic	M23A	Circular	Flat and extended	Plicate	Cottony	Light gray	Gray	Septate	Not observed
Asymptomatic	M27A	Filamentous	Flat and extended	Plicate	Velvety	White w. gray center	Light gray	Septate	Not observed
Asymptomatic	M29A	Rizoidal	Flat and extended	Sectorized	Cottony	Light gray	Light gray	Septate	Not observed
Asymptomatic	M30A	Irregular	Elevated and limited	Sectorized	Cottony	Light gray	Gray	Septate	Not observed
Asymptomatic	M31	Irregular	Umbilicated convex	Sectorized	Cottony	Light gray	Black	Septate	Not observed
Asymptomatic	M32	Irregular	Elevated and limited	Sectorized	Velvety	White	Light brown	Septate	Not observed
Asymptomatic	M33	Irregular	Elevated and limited	Sectorized	Velvety	White	Light brown	Septate	Not observed
Asymptomatic	M34	Rizoidal	Umbilicated convex	Plicate	Velvety	Light gray	Gray	Septate	Not observed
Asymptomatic	M8A	Circular	Flat and extended	Sectorized	Cottony	White	Light gray	Septate	Not observed
Monoculture	M11S	Circular	Elevated and limited	Cerebriform	Pulverulent	Beige	Cream	Septate	Not observed
Monoculture	M12S	Irregular	Umbilicated convex	Sectorized	Pulverulent	White w. light orange	Light orange	Non-septate	Phragmospores
Monoculture	M13S	Circular	Elevated and limited	Plicate	Velvety	Light gray	Brown	Septated	Not observed
Monoculture	M16S	Circular	Elevated and limited	Plicate	Velvety	Black w. white fruitbodies	Black	Septated	Oval spores
Monoculture	M25S	Circular	Flat and extended	Plicate	Pulverulent	Gray with brown edges	Black	Single cells	diophore with mucous sp
Monoculture	M28S	Circular	Elevated and limited	Sectorized	Cottony	Violet	Violet	Septated	Not observed
Monoculture	M37S	Circular	Elevated and limited	Sectorized	Velvety	Light brown	Light brown	Septated	Oogonio
Monoculture	M41S	Circular	Flat and extended	Sectorized	Cremona	Pink	Pink w. gray ring	Yeast	Not observed
Monoculture	M42S	Circular	Elevated and limited	Plicate	Velvety	White	Cream	Septated	Not observed
Monoculture	M43S	Irregular	Elevated and limited	Radial grooves	Cottony	Light gray	Cream	Septated	Scotescospores
Monoculture	M44S	Irregular	Flat and extended	Cerebriform	Cottony	Light brown	Dark brown	Haustorio	Not observed
Monoculture	M46S	Circular	Flat and extended	Plicate	Velvety	Gray	Black	Septated	diophore with mucous sp
Monoculture	M47S	Circular	Elevated and limited	Sectorized	Cottony	Light brown	Cream	Septated	Not observed
Monoculture	M50S	Filamentous	Flat and extended	Radial grooves	Pulverulent	Gray	Cream	Septated	Scotescospores
Monoculture	M52S	Circular	Flat and extended	Cerebriform	Pulverulent	White	Cream	Septated	Not observed
Monoculture	M53S	Circular	Flat and extended	Sectorized	Pulverulent	white	Cream	Septated	Arthroconidia
Monoculture	M61S	Irregular	Elevated and limited	Plicate	Cottony	White	Cream	Septated	Not observed
Monoculture	M62S	Circular	Flat and extended	Plicate	Velvety	Dark gray	Black	Septated	Sessile round spores
Monoculture	M65S	Circular	Flat and extended	Plicate	Velvety	Light brown	Light brown	Septated	Didymospore
Monoculture	M66	Irregular	Umbilicated convex	Sectorized	Pulverulent	Light orange	Light orange	Non-septate	Phragmospores
Monoculture	M95	Circular	Flat and extended	Plicate	Cottony	Dark brown	Black	Septated	Not observed
Polyculture	M10S	regular	Umbilicated convex	Plicate	Cottony	Black	Black	Septated	Not observed
Polyculture	M1S	Circular	Flat and extended		Velvety	Gray	Black	Septated	Not observed
Polyculture	M24S	Irregular	Umbilicated convex	Sectorized	Pulverulent	Light orange	Light orange	Non-septate	Phragmospores
Polyculture	M2S	Irregular	Umbilicated convex	Sectorized	Pulverulent	Light orange	Light orange	Non-septate	Phragmospores
Polyculture	M38S	Irregular	Umbilicated convex	Sectorized	Pulverulent	Light orange	Light orange	Non-septate	Phragmospores
Polyculture	M39S	Irregular	Flat and extended	Plicate	Pulverulent	White	Gray	Septated	Oogonio
Polyculture	M3S	Circular	Flat and extended		Velvety	Gray	Black	Septated	Not observed
Polyculture	M40S	Irregular	Umbilicated convex	Sectorized	Pulverulent	Light orange	Light orange	Non-septate	Phragmospores
Polyculture	M49S	Irregular	Elevated and limited	Sectorized	Cottony	White	Cream	Septated	Not observed
Polyculture	M4S	Irregular	Umbilicated convex	Sectorized	Pulverulent	Light orange	Light orange	Non-septate	Phragmospores
Polyculture	M58S	Circular	Umbilicated convex	Sectorized	Cottony	White	Cream	Septated	Not observed
Polyculture	M5S	Irregular	Umbilicated convex	Sectorized	Pulverulent	Light orange	Light orange	Non-septate	Phragmospores
Polyculture	M63S	Irregular	Elevated and limited	Sectorized	Pulverulent	White	Cream	Rhizoids	Oogonio
Polyculture	M7S	Irregular	Umbilicated convex	Sectorized	Pulverulent	Light orange	Light orange	Non-septate	Phragmospores
Restoration	M14S	Circular	Elevated and limited	Sectorized	Cottony	White	White	Septated	Not observed
Restoration	M15S	Circular	Flat and extended	Sectorized		Light brown	Dark brown	Septated	Not observed
Restoration	M20S	Circular	Flat and extended	Sectorized	Pulverulent	Gray	Light grey	Septated	Sitroform spores
Restoration	M21S	Filamentous	Flat and extended	Plicate	Velvety	Pink	Pink	Septated	Mucous spores
Restoration	M25S	Irregular	Elevated and limited	Cerebriform	Velvety	White	Cream	Non-septate	Astrospheres
Restoration	M36S	Circular	Flat and extended	Sectorized	Pulverulent	Gray	Dark brown	Non-septate	Conidiosporangium
Restoration	M54S	Circular	Flat and extended	Plicate	Cottony	Light gray	Pink	Septated	Chlamydiospore
Restoration	M55S	Circular	Flat and extended	Plicate	Cottony	Light gray	Light pink	Septated	Not observed
Restoration	M56S	Circular	Convexa y umbilicada	Cerebriform	Cottony	White	Beige	Non-septate	Sporangium
Restoration	M57S	Circular	Flat and extended	Cerebriform	Pulverulent	White	White	Non-septate	Not observed
Restoration	M60S	Circular	Flat and extended	Plicate	Pulverulent	White	Beige	Non-septate	Aleuriospore
Restoration	M64S	Circular	Flat and extended	Plicate	Velvety	Gray	Black	Septated	ogonium with antheridiu