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Artículo científico

Fungal communities isolated from symptomatic naranjilla (*Solanum quitoense* Lam.) leaves in the Ecuadorian Amazon region

Comunidades fúngicas aisladas de hojas sintomáticas de naranjilla (*Solanum quitoense* Lam.) en la región amazónica ecuatoriana

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ABSTRACT.- Fungal isolates were recovered from symptomatic naranjilla *Solanum quitoense* Lam. leaves sampled in the Pastaza province, an important productive region in the eastern Ecuadorian Amazon. Filamentous fungi were identified with molecular methods based on ITS sequencing. Among 68 recorded fungi, the isolation of mycobiota resulted in 14 taxa. *Diaporthe citri, D. schini,* and *Xylaria berteri* were the most frequently isolated species. To our knowledge, more than 90% of the taxa recovered in this study are reported for the first time in *S. quitoense*. Diversity indices suggest that symptomatic tissues represent a moderately diverse reservoir for fungal communities. This is the first study to characterize fungal diversity associated with symptomatic naranjilla leaves showing necrosis or chlorosis tissues through ITS sequencing. The findings presented in this study are relevant, as they can assist in the isolation of a great number of fungal taxa, which could potentially harbor a broad diversity of plant-derived natural molecules with biologically active substances.

KEYWORDS: Endophytic fungi, ITS-sequencing, Lulo, Mycobiota, rDNA

RESUMEN.- Se recuperaron aislados de tejidos sintomáticos de hojas de naranjilla *Solanum quitoense* Lam. en la provincia de Pastaza, una zona de producción importante de la región amazónica del Ecuador. Se identificaron hongos filamentosos por medio de técnicas moleculares basados en la secuencia de la región ITS. De un total de 68 hongos, se identificaron 14 taxa. La mayor cantidad de registros pertenecieron a las especies *Diaporthe citri, D. schini*, y *Xylaria berteri.* Según lo que conocemos, más del 90% de los taxones recuperados en este estudio se reportan por primera vez en *S. quitoense*. Los índices de diversidad sugieren que los tejidos sintomáticos representan un reservorio moderadamente diverso para comunidades de hongos. Este estudio es el primero en caracterizar la diversidad fúngica asociada con hojas sintomáticas que presentan tejidos necróticos o cloróticos en naranjilla. Los resultados de este estudio son relevantes, ya que aportan a la obtención de un gran número de especies de hongos que, potencialmente, podrían albergar una gran diversidad de moléculas naturales derivadas de plantas con sustancias de actividad biológica.

PALABRAS CLAVES: ADNr, Hongos endófitos, ITS, Lulo, Micobiota

INTRODUCTION

The naranjilla plant, or lulo (*Solanum quitoense* Lam.), is a tropical Solanaceae widely consumed in Peru, Ecuador, Colombia and Central America and in high demand at agro-industrial markets throughout the region (Flórez et al. 2008; Pulido et al. 2008; Acosta et al. 2009; Granados et al. 2013). The crop is native to the Andean foothills and has highly ramified cylindrical stems, with large oval shaped leaves (León 2000). In spite of its natural mechanical defenses (e.g., spine-covered leaves) and the use of fertilizers, fungicides, and bio-control agents (Revelo et al. 2010), a series of studies report a broad list of pathogenic fungi, such as *Phytophthora infestans, Sclerotinia sclerotiorum, Fusarium oxysporum, Colletotrichum spp. and Cladosporium spp.*, which cause several detrimental diseases that affect naranjilla (Montes-Rojas et al. 2010; Revelo et al. 2010). There is a wide array of

farmer-selected varieties of the naranjilla (Casierra-Posada et al. 2004), and most cultivation practices (e.g., sanitary pruning) rely on empirical approaches based on local knowledge, with no experimental research support to fully understand and control plant pathogens (Montes-Rojas et al. 2010; Revelo et al. 2010).

Fungal communities have adapted to an extensive array of environments with distinctive physiological requirements that impact species diversity and colonization (Petrini 1996; Schadt et al. 2003; Opik et al. 2006; Das et al. 2007; Mondal et al. 2007; Frohlich-Nowoisky et al. 2009). To date, fungi inhabiting vegetative tissues have been isolated from a considerable variety of plant species, in both temperate (Jumpponen and Jones 2009; Sánchez-Márquez et al. 2010; Ghimire et al. 2011; Gonzalez and Tello 2011; Koukol et al. 2012) and tropical regions (Frohlich et al. 2000; Photita et al. 2001; Suryanarayanan et al. 2005; Arnold and Lutzoni 2007; Thomas et al. 2008; Gazis and Chaverri 2010; Vega et al. 2010; Rivera-Orduña et al. 2011; Vaz et al. 2014); however, their ecological roles and abundance may vary depending on the host or tissue they are found to inhabit (Johnston et al. 2006; Sieber 2007).

Fungal microorganisms that colonize healthy plant tissues without causing adverse symptoms have been defined as endophytes (Carroll 1986; Petrini 1991; Hallmann et al. 1997; Hyde and Soytong 2008); however, previous studies have shown that apparently benign species may subsequently shift their ecological role to pathogens or saprobes due to environmental stress factors (e.g., nutrient deficiency, drought, heat) (Photita et al. 2004; Agrios 2005; Cakmak 2005; Promputtha et al. 2007). For example, latent pathogens of the genus *Colletotrichum* are known to live innocuously within healthy tissues prior to showing of symptoms (Cerkauskas 1988; Prihastuti et al. 2009), while an endophytic stage might be of relevant importance within the life cycle of tropical pathogens (for example, *Deightoniella torulosa in Musa acuminata* Photita et al. 2004). On the other hand, the mycobiota is likely to vary depending on the host (Arnold and Herre 2003). For instance, some species of the genus *Diaporthe* may act as pathogens in some hosts (Santos et al. 2011), and as endophytes in others (Sebastianes et al. 2011). Hence, the impact of fungal communities on plant species may be overlooked (Omacini et al. 2001).

Our understanding of fungal communities has benefited from an increasing number of fungal strains and species isolated from tropical crops of economic importance (Zhu et al. 2000; Sánchez-Márquez et al. 2007). Among plant aerial tissues, diseased leaves showing necrotic symptoms or chlorotic tissues represent a vulnerable interface between plants and their surroundings, where complex fungi communities grow within ever-changing environments (Arnold et al. 2003). In particular, changes within the inner biochemical milieu increase the vulnerability of symptomatic leaves as potential reservoirs for high fungal assemblages through wounds (Schulz and Boyle 2006). Recent studies show that several species within the mycobiome might be isolated from both dead and living plant material (U'Ren et al. 2010; Osono and Hirose 2011; Yuan and Chen 2014; U'Ren and Arnold 2016). Yet, while previous studies have used molecular characters to identify fungal communities inhabiting tropical plants (Crozier et al. 2006; Sieber 2007; Rungjindamai et al. 2008; Thomas et al. 2008; Gazis and Chaverri 2010; Mohamed et al. 2010; Abreu et al. 2012; Vaz et al. 2014), little is known about the fundamental aspects derived from ecological approaches in fungi associated with symptomatic foliar tissues.

In light of the growing interest in the study of microbial communities and interspecific associations in plant biology (Backman and Sikora 2008), and considering that necrotic lesions are detrimental to the fruit production demanded for agro-industrial crops (De Lucca 2007), this study aims to identify the fungal community inhabiting symptomatic foliar tissues of *S. quitoense* showing necrotic symptoms or chlorotic tissues, collected in the eastern Ecuadorian Amazon region. The study of microbial fungi in diseased leaves is, in turn, expected to present an alternative to isolate native fungal species with potential medicinal and pharmaceutical properties.

MATERIALS AND METHODS

Study site and sample collection.- This research was conducted in plantations of a wild variety of naranjilla (*S. quitoense* cv. *quitoense*) in the Pastaza province, in the eastern Amazon region of Ecuador (1°28′ 12.821" S, 72°5′45.986" W, 1115 m.a.s.l.). Mature plants of about 1–2 m in height were randomly sampled when precipitations reached their first annual peak during May of 2015, in a 1 hectare crop. One leaf per plant showing symptomatic tissues with necrosis was collected. Twenty samples were transported to the laboratory in sterile plastic bags and stored for 24 to 48 h at 4 °C until the fungi was isolated (Agrios 2005).

Isolation of fungi.- In total, four leaf segments (1 × 1 cm) per leaf were taken from symptomatic tissue and were used for the isolation of fungi according to protocols modified from the method described by Crous et al. (2009). To induce sporulation, samples were surface-disinfected through immersion in 70% ethanol (1 min) and 10 % sodium hypochlorite (5 min). Samples were washed

in sterile water three times before transferring leaf segments to Petri dishes (90 mm) containing a sterile Potato Dextrose Agar (PDA, Difco Laboratories, Detroit, MI) supplemented with chloramphenicol 30 ppm to suppress bacterial proliferation, following Crous et al. (2009). The plates were incubated at 20–27 °C (12-h light/12-h dark regime) and checked regularly for fungal growth for two weeks. After hyphal tips emerged from the leaf tissue, different mycelium fragments were subcultured onto new Petri dishes with the same media, for further purification and identification (Crozier et al. 2006). Filamentous fungal isolates were morphologically evaluated and divided into morphotypes based on macroscopic observation of colonies and microscopic identification of reproductive structures such as distinctive spores (Crous et al. 2009)

DNA extraction.- About 40 mg of fresh fungal tissue was disrupted using a sterile blade, and genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI) in accordance with manufacturer's instructions. DNA concentration was quantified with a spectrophotometer (Nanodrop Technologies, Rockland, Denmark) and stored at -20 ºC.

PCR amplification.- The DNA was amplified using primer pairs for the internal transcribed spacer region (ITS) of rDNA, ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′), commonly used for fungal barcoding (White et al. 1990), and GoTaq Green Master mix (Promega, Madison, WI) according to manufacturer's protocol. Reactions were carried out in 25 µl final volume under the following conditions: the initial denaturation step at 94 °C for 5 min, followed by 35 cycles at 94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, and a final elongation at 72 °C for 10 min. All amplifications performed included a positive control containing 3 µl of fungal DNA and a negative control containing 1.5 µl of master mix. PCR products were visualized with 1.5 µl of 100 bp ladder by electrophoresis on a 2.5 % (w/v) agarose gel containing Sybr Safe (Invitrogen, Mulgrave, Australia) under UV light. Amplification products were sequenced at a commercial laboratory (Macrogen, Seoul, South Korea).

Fungal identification.- To identify the fungal isolates based on ITS sequence, the consensus sequences were subjected to the BLASTn search with the National Center for Biotechnology Information (NCBI, Bethesda, MD) database. Multiple sequence alignment was performed using CLUSTAL W version 1.8 (Thompson et al. 1994). Query coverage and identities were calculated using MEGA Version 6.0 (Tamura et al. 2013). Fungal species were defined using an ITS region identity threshold of 98% to sequences deposited in GenBank. All ITS sequences obtained in this work were deposited in GenBank under the accession numbers: MF185318–MF185359. Fungal classification follows the MycoBank (http://www.mycobank.org) database.

Statistical analysis.- Colonization frequency (CF) was calculated as the number of discs colonized by a given fungus divided by the total number of discs in that sample, expressed as percentages following the method of Hata and Futai (1995). Relative frequency of isolation (RF) was determined as the total number of discs colonized by a given taxon divided by the total number of taxa obtained from molecular analyses. Fungal species diversity was estimated using PAST, version 1.9 (Paleontological Statistics) software (Hammer et al. 2001) considering Simpson's index to quantify species dominance (Simpson 1949), Shannon's index to estimate species diversity (Shannon and Weaver 1963), and Margalef's index to assess species richness using the following formulas:

1. Simpson's diversity (1-D) = $1 - \Sigma(pi)^2$
2. Shannon-Wiener index (H') = $-\Sigma pi\ (\log_2 p_i)$
3. Margalef's (D^{mg}) = (S-1) / ln N

where, pi was the proportion of colonization frequency of a given taxon in diseased tissue samples, S is the total number of taxa isolated from diseased foliar tissue samples, and N is the number of species recorded.

RESULTS

A total of 68 fungal isolates were recovered from symptomatic foliar tissues of *S. quitoense*. Isolated fungi belonged to the phyllum Ascomycota, distributed in four orders: Diaporthales, Glomerellales, Sordariales, and Trichosphaeriales. Fungal isolates comprised four families, six genera and 14 identified taxa based on molecular methods (Table 1). Query coverage ranged from 93-99% within our sampled fungal community, except from a single isolate of a possibly *Xylaria* species, which scored 88%. Fungal communities presented a low overall isolation rate of 5.75%. The most frequently isolated fungi were *Diaporthe citri, D. schini*, and *Xylaria berteri,* which accounted for the highest relative frequency, and each presented a ≥ 10% isolation rate (Table 2). Among the total fungal isolates, four taxa (28.5%) occurred as singlets (≤ 2 isolates), including *Diaporthe, Nemania,* and *Phomopsis* colonies. *Diaporthe, Phomopsis,* and *Xylaria* were the most frequently recovered genera (Table 3). Diversity indices displayed high values regarding species dominance (1-D = 0.894), diversity (H' = 3.376), and richness (D^{mg} = 3.07).

Table 1. ITS-based identification of fungal isolates recovered from symptomatic leaves of Solanum quitoense in the Ecuadorian Amazon.

Isolate	Proposed fungal taxon[a]	GenBank Accesion No.	QC (%)	Id (%)	Sequence length (bp)	[b]Closest species in GenBank	Reference
L5N29	*Colletotrichum sp.*	MF185323	99	100	581	*Colletotrichum sp.* GM77 (KC512139.1)	Álvarez et al. 2014
L5N80		MF185335	99	100	584		
L5N4		MF185318	99	99	582		
L5N47	*Diaporthe citri* F.A. Wolf	MF185327	99	100	577	*Diaporthe citri* (KC343051.1)	Gomes et al. 2013
L5N67		MF185330	99	100	577		
L5N76		MF185332	99	100	579		
L5N94		MF185339	93	100	653		
L5N127		MF185344	99	100	579		
L5N131		MF185345	98	100	583		
L5N133		MF185346	99	100	582		
L5N134		MF185347	99	100	579		
L5N135		MF185348	98	100	591		
L5N138		MF185350	99	100	579		
L5N142		MF185351	99	100	579		
L5N163		MF185357	99	100	577		
L5N174		MF185358	99	100	577		
L5N24	*Diaporthe melonis* Beraha & M.J. O'Brien	MF185322	99	100	572	*Diaporthe melonis* (NR_103700.1)	Gomes et al. 2013
L5N151		MF185355	99	100	580		
L5N37	*Diaporthe phaseolorum* (Cooke & Ellis) Sacc.	MF185324	99	100	578	*Diaporthe phaseolorum* (KP182395.1)	Santos et al. 2015
L5N143	*Diaporthe phaseolorum* (Cooke & Ellis) Sacc.	MF185352	99	99	581		
L5N8	*Diaporthe schini* R.R. Gomes, C. Glienke & Crous	MF185319	99	100	575	*Diaporthe schini* (NR_111861.1)	Gomes et al. 2013
L5N71	*Diaporthe schini* R.R. Gomes, C. Glienke & Crous	MF185331	99	99	577		
L5N10	*Diaporthe sp.* 2	MF185320	99	100	576	*Diaporthe sp.* 1 RG-2013 (KC343203.1)	Gomes et al. 2013
L5N11		MF185321	99	100	577		
L5N40	*Diaporthe terebinthifolii* R.R. Gomes, C. Glienke & Crous	MF185325	99	100	575	*Diaporthe terebinthifolii* (NR_111862.1)	Gomes et al. 2013
L5N101	*Nemania diffusa* (Sowerby) Gray	MF185340	98	100	582	*Nemania diffusa* (AB625422.1)	Okane et al. 2008
L5N63	*Neurospora tetrasperma* Shear & B.O. Dodge	MF185329	99	100	587	*Neurospora tetrasperma* (AY681194.1)	Cai et al. 2006
L5N77		MF185333	99	100	589		
L5N87	*Phomopsis asparagi* (Sacc.) Grove	MF185336	99	100	577	*Phomopsis asparagi* (KR812246.1)	Reis et al. 2015
L5N92		MF185338	99	100	580		
L5N104		MF185342	99	100	585		
L5N146		MF185354	99	100	578		
L5N91	*Phomopsis sp.* 1	MF185337	98	100	597	*Phomopsis sp.* CML 1315 (JN153055.1)	Abreu et al. 2012
L5N159	*Phomopsis sp.* 2	MF185356	99	100	578	*Phomopsis sp.* NR-2006-D53 (DQ480356.1)	Phongpaichit et al. 2006
L5N41	*Phomopsis sp.* 3	MF185326	98	100	576	*Phomopsis sp.* Vega394 (EU002915.1)	Vega et al. 2010
L5N79		MF185334	97	100	589		
L5N102		MF185341	98	100	577		
L5N185		MF185359	98	100	578		
L5N49	*Xylaria berteri* (Mont.) (Mont.) Cooke ex J.D. Rogers & Y.M. Ju	MF185328	99	100	592	*Xylaria berteri* (KP133344.1)	nd
L5N105		MF185343	88	100	583		
L5N144	*Xylaria berteri* (Mont.) (Mont.) Cooke ex J.D. Rogers & Y.M. Ju	MF185353	98	100	592	*Xylaria berteri* (GU324749.1)	Hsieh et al. 2010

[a] Taxonomic identification of fungal isolates was conducted using amplification of ITS4 and ITS5 by PCR. All sequences were deposited in the NCBI GenBank (Accession No. column). QC: query cover, Id: identity. b Sequences available in GenBank used for comparisons with sequences obtained in this study via BLAST software.

Table 2. Total number of isolates, isolation rate (%) and relative frequency of ascomycetous fungi recovered from symptomatic foliar tissues of *Solanum quitoense* in the Ecuadorian Amazon.

Taxa	No. isolates	Isolation rate (%)	Relative Frequency
Diaporthales			
Diaporthe citri	15	18.75	1.0000
Diaporthe melonis	4	5	0.2667
Diaporthe phaseolorum	5	6.25	0.3333
Diaporthe schini	10	12.5	0.6667
Phomopsis asparagi	5	6.25	0.3333
Phomopsis sp. 3	4	5	0.2667
Glomerellales			
Colletotrichum sp.	3	3.75	0.2000
Sordariales			
Neurospora tetrasperma	5	6.25	0.3333
Trichosphaeriales			
Xylaria berteri	10	12.5	0.6667

Rare taxa (≤ 2 isolates): *Diaporthe sp*. 1, *Diaporthe sp.* 2, *Diaporthe terebinthifolii*, *Phomopsis sp.* 1, *Phomopsis sp.* 2, and *Nemania diffusa*.

DISCUSSION

In the Tropics, research on fungi associated with the plant's internal tissues has gained greater attention, considering that ecological relationships with photosynthetic organisms comprise a wide variety of fungal species (Petrini 1991; Bills 1996; Saikkonen et al. 2004; Arnold and Lutzony 2007; Oono et al. 2015). Previous works have reported Ascomycota as the dominant phyllum within fungi inhabiting foliar plant tissues (Arnold and Lutzoni 2007; Vaz et al. 2014). This work represents the first attempt to characterize fungal diversity associated with symptomatic naranjilla foliar tissue using molecular approaches through ITS sequencing. Typically, the identification of fungal species lacks morphological variation, while in molecular approaches, the amplification of commonly used regions (e.g. mitochondrial cytochrome c oxidase subunit 1) is often complicated and not variable enough (Crouch et al. 2017). However, previous studies on fungal taxonomy have proposed the ITS region as the primary fungal barcode marker (Schoch et al. 2012) given its high probability for correctly identifying fungal species through a measure of genetic distances (Del-Prado et al. 2010). We identified 14 ascomycetous fungal taxa. Results showed that *D. citri, D. schini,* and *X. berteri* were the species most frequently isolated from symptomatic foliar tissues of naranjilla in the eastern Ecuadorian Amazon region. Recent studies based on ITS sequencing have been shown to yield misleading species diagnoses in fungi associated with grass, particularly for the *Colletotrichum* species complex. The latter might explain the unknown *Colletotrichum* taxa isolated in this study. We strongly recommend the use of metagenomic and small-subunit RNA-based sequence analysis techniques for future research, as this has previously been validated as an accurate estimator of microbial assessments when evaluating species richness and evenness (Fierer et al. 2007).

In previous studies, several pathogenic fungal species have been reported in cultivated and wild naranjilla such as *Botrytis sp., Cercospora sp., Cladosporium sp., Colletotrichum spp., Fusarium oxysporum, Phytophthora infestans, Rhizoctonia solani, Sclerotinia*

Table 3. Number of isolates and species per genus recovered in symptomatic leaves of *Solanum quitoense* in the Ecuadorian Amazon

Genus	No. isolates	No. Species
Colletotrichum	3	1
Diaporthe	37	6
Neurospora	5	1
Nemania	1	1
Phomopsis	12	4
Xylaria	10	1

sclerotiorum, and *Septoria solanicola* (Rogg 2000; Quinchia and Cabrera 2006; Montes-Rojas et al. 2010; Revelo et al. 2010). from which only *Colletotrichum* colonies were identified in our work. To our knowledge, nine taxa isolated in this study, *D. citri, D. melonis, D. phaseolorum, D. schini, D. terebinthifolii, Phomopsis asparagi, Neurospora tetrasperma, Nemania diffusa,* and *X. berteri,* have not previously been reported for *S. quitoense*. Also, isolates of *Diaporthe* (n=2) and *Phomopsis* (n=3) could not be identified to the species level. Both of these genera comprise highly complex taxa with a considerable amount of cryptic species that have yet to be described (Gomes et al. 2013). In this study, one isolate (L5N105) proved to be genetically distinct from its closest match in GenBank database. Although the latter may suggest that these isolates could represent unknown species, further morphological and molecular assessments are needed.

Fungal communities are known to have high species diversity in the tropics, as the incidence of fungi within foliar tissues tends to increase with decreasing latitude (Arnold and Lutzoni 2007). In our study, fungal species found within symptomatic naranjilla tissues showed a low overall isolation rate. This may be due to the sampling period, as the aerial tissues of plants may be less vulnerable to internal colonization when heavy rains and high humidity occur (Photita et al. 2001). Our results coincide with previous work on other fungal communities in tropical plants (Frohlich et al. 2000; Arnold and Lutzoni 2007), where fungal assemblages have been described to show a pattern of few common species and a dominance by rare species. While the current study shows high values of species dominance and diversity and richness among the fungal communities found within diseased foliar tissues of naranjilla plants, our results may not show the most common fungal species, but rather colonies that grow rapidly under the isolation methods applied in this study, such as the PDA medium and the 12-h light/12-h dark regime (Arnold and Lutzoni 2007; Sánchez-Márquez et al. 2007).

Diversity indices calculated in this work suggest that leaves presenting necrotic lesions may in fact represent a moderately rich ecosystem for fungal communities (1-D = 0.894; H' = 3.376; D^{mg} = 3.07). Several of the fungal species recovered here, including *Diaporthe spp.* (Zhang et al. 1999; Santos et al. 2011; Sebastianes et al. 2012), *Phomopsis spp.* (Zhang et al. 1999; Koh et al. 2005) and *Colletotrichum sp.* (Hyde et al. 2009; Wikee et al. 2011; Cannon et al. 2012) have been previously reported as pathogens from many plant taxa. However, we cannot assure the ecological role of the fungi isolated here, as we did not assess if isolates were acting as endophytes, saprobes, or parasites given that the natural behavior of fungal species may be influenced by environmental conditions and/or interspecific relationships (Millar 1980; Viret and Petrini 1994; Williamson 1994; Wilson 1995; Promputtha et al. 2007). In addition to this, a great number of fungal species may act as secondary colonizers of necrotic leaf tissues, including soil saprophytes (Aegerter et al. 2000; Bruez et al. 2014; Peay et al. 2016). We recommend conducting Koch's postulates in future studies to determine fungal phytopatogenecity activity.

In general, although the majority of fungal community studies have focused on endophytes isolated from asymptomatic tissues, this work identifies fungal organisms capable of surviving within symptomatic leaves. The findings presented in this study are relevant because they can lead to assisting in the isolation of a great number of fungal taxa, which could potentially harbor a broad diversity of plant-derived natural molecules with biologically active substances (Schulz et al. 2002; Kharwar et al. 2011). Recent studies confirm a growing interest in isolating mycobiota for novel compounds (Dreyfuss and Chapela 1994; Dettrakul et al. 2003; Kobayashi et al. 2003; Kumar and Hyde 2004; Tejesvi et al. 2007). Furthermore, based on the premise that plant-fungi interactions have evolved over the course of a long evolutionary process, some fungal species have been shown to mimic the host compounds in the metabolites produced (Strobel 2002). Further studies on the chemical composition and medicinal properties of the mycobiome in *S. quitoense* are required to determine their potential in producing bioactive metabolites. The latter may, in turn, be relevant to the biocontrol agents of other fungal phytopatogens by contending for the same ecological niche (Asiegbu et al. 2005; Brum et al. 2012).

We highly encourage the direct sequencing of leaves as a culture-free method to assess species diversity and composition in future studies (Arnold 2007), given that the fungal diversity of symptomatic tissues of *S. quitoense* may be greater than that reported here. Furthermore, isolating fungal microbes from other aerial tissues (e.g. stems, flowers, fruits) and comparing the different varieties of naranjilla cultivars is also encouraged. Even though symptomatic leaves may act as potential reservoirs for latent pathogens (Andersen and Walker 1985; Schulz and Boyle 2006), the modes of inter-specific interactions and the pathogenicity of fungal species remains to be demonstrated. A comparison between both symptomatic and asymptomatic tissues might provide a broader perspective regarding the diversity succession of fungal species when diseased leaves occur.

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