Endophytic Fungi

Subjects: Microbiology
Contributor: Laura Gioia

An extensive literature search was performed to review current knowledge about endophytic fungi isolated from plants included in the European Food Safety Authority (EFSA) dossier. The selected genera of plants were *Acacia*, *Albizia*, *Bauhinia*, *Berberis*, *Caesalpinia*, *Cassia*, *Cornus*, *Hamamelis*, *Jasminus*, *Ligustrum*, *Lonicera*, *Nerium*, and *Robinia.* A total of 120 fungal genera have been found in plant tissues originating from several countries. *Bauhinia* and *Cornus* showed the highest diversity of endophytes, whereas *Hamamelis*, *Jasminus*, *Lonicera*, and *Robinia* exhibited the lowest. The most frequently detected fungi were *Aspergillus*, *Colletotrichum*, *Fusarium*, *Penicillium*, *Phyllosticta*, and *Alternaria.* Plants and plant products represent an inoculum source of several mutualistic or pathogenic fungi, including quarantine pathogens. Thus, the movement of living organisms across continents during international trade represents a serious threat to ecosystems and biosecurity measures should be taken at a global level.

Keywords: endophytic fungi ; crop protection ; Acacia ; Albizia ; Bauhinia ; Berberis ; Caesalpinia ; Cassia ; Cornus ; Hamamelis ; Jasminus ; Ligustrum ; Lonicera ; Nerium ; Robinia

1. Introduction

Endophytic fungi are ubiquitous to plants, and are mainly members of Ascomycota or their mitosporic stage, but they also include some taxa of Basidiomycota, Zygomycota, and Oomycota. Endophytes are organisms living within the tissues of plants [1] establishing stable relationships with their host, ranging from non-pathogenic to beneficial [2][3]. The endophytic fungi communities represent an enormous reserve of biodiversity and constitute a rich source of bioactive compounds used in agriculture [4][5]. For these reasons, they have attracted the attention of the scientific community worldwide. By definition, all or at least a significant part of the endophytic fungi life cycle occurs within the plant tissues without causing symptoms to their host [6][7][8]. A wide range of fungi, including pathogens and saprophytes, may be endophytes. Several pathogens live asymptomatically within plant tissues during their latency or quiescent stage, while some saprobes can also be facultative parasites [1][8][9]. Fungal endophytes are influenced by abiotic and biotic factors, occupying different habitats and locations during their life cycle phases. Even if host plants do not show any symptoms, they may represent a source of inoculum for other species [10][11][12][13]. Furthermore, changes in environmental conditions or species hosts may modify the fungal behavior, thus producing disease symptoms [8][11][14]. Large quantities of plants and plant material that are globally traded might contain asymptomatic infections of these fungi. It is generally accepted that the movement of plants and plant products by global trade and human activities is the most common way to introduce exotic pathogens and pests in non-endemic countries. Plant health is increasingly threatened by the introduction of emerging pests and/or pathogens [15][16]. Noticeable examples are represented by the invasion of alien plant pathogens into new areas [17][18][19]. Generally, biological invasions are the main threat to biodiversity [20], causing a decrease in species richness and diversity [20][21] or affecting local biological communities [22], as well as changing ecosystem processes [23][24][25].

In this scenario, the European Food Safety Authority (EFSA) Panel on Plant Health is responsible for the risk assessment, evaluations of risk reduction options, as well as guidance documents [26] in the domain of plant health for the European Union (EU) [26][27]. Commission Implementing Regulation (EU) [28] prohibits the importation of 35 so-called ‘High-Risk Plants, plant products and other objects’ from all third (non-EU) countries as long as no full risk assessment has been carried out. The EFSA Panel on Plant Health was requested to prepare and deliver risk assessments for these commodities [27][28], to evaluate whether the plant material will remain prohibited or removed from the list, with or without the application of additional measures [27][29]. The Commodity Risk Assessment has to be performed on the basis of technical dossiers provided by National Plant Protection Organizations of third countries. Information required for the preparation and submission of technical dossiers includes data on the pests potentially associated with the plant species or genera and on phytosanitary mitigation measures and inspections [30][31].

These plants have been identified as ‘High-Risk Plants’ by the EU since they ‘host commonly hosted pests known to have a major impact on plant species which are of major economic, social or environmental importance to the Union’ [28]. However, among these 35 plant genera, within the meaning of Art. 42 of Regulation (EU) 2016/2031, a list of only 13 taxa have been selected by the EFSA as plants mostly traded for ornamental purposes. According to this list, we have reviewed the following genera: *Acacia* Mill., *Albizia* Durazz., *Bauhinia* L., *Berberis* L., *Caesalpinia* L., *Cassia* L., *Cornus* L., *Hamamelis* L., *Jasminus* L., *Ligustrum* L., and *Robinia* L. In this article, as much as possible, we highlight the potential risks associated with the movement of plants or materials among nations. Although other plant species may also have a significant impact, this review is limited to plants included in EU regulation [28] that do not originate within Europe. Thus, given these perspectives for future assessments, the present investigation offers an up-to-date snapshot of endophytic fungi associated with the so-called ‘High-Risk Plants for ornamental purpose’. The aim is to facilitate the information required for technical dossiers, needed by the EFSA to perform the Commodity Risk Assessment of 13 plants mandated on an EU import list.

2. An Overview of Fungal Diversity and Frequency

Investigations on the mycobiota of plants frequently reported new taxa or new species distribution, and several fungi are still undiscovered or undetected. Numerous higher plants have developed a variety of resistance mechanisms to prevent fungal infections. However, the presence of weakly pathogenic fungi in healthy plant tissues highlights the evolutionary continuum between latent pathogens and symptomless endophytes [15]. Generally, all plants have symbiotic interactions with fungal endophytes which can influence host performance in terms of disease resistance [32][33][34], stress tolerance [35], and biomass accumulation [36]. Fungal endophytes may also change according to plant tissues colonized [37], phenological growth stages, host genotypes [38], and geographical distribution areas [39].

In this review, a total of 428 endophytic species belonging to 122 fungal genera have been found in association with 13 plant genera (Table 1). The greatest level of fungal diversity was reported in in association with *Bauhinia* with 43 fungal genera and 94 fungal species, and *Cornus* with 44 fungal genera and 78 fungal species. The degree of fungal recovery from *Acacia* (29 genera, 51 species), *Albizia* (14 genera, 27 species), *Berberis* (17 genera, 29 species), *Caesalpinia* (19 genera, 42 species), *Cassia* (15 genera, 19 species), *Ligustrum* (20 genera, 29 species), and *Nerium* (21 genera, 37 species) was nearly half in comparison to the abundance noted in the genera *Bauhinia* and *Cornus*. Nonetheless, the lowest diversity showed for *Hamamelis* (4 species/genera), *Jasminus* (7 species, 1 genera), *Lonicera* (3 species/genera), and *Robinia* (6 species/genera) was also due to the lack of published research about fungal endophytes in these plant genera.

Table 1. Endophytic fungi isolated from *Acacia* (AC), *Albizia* (AL)., *Bauhinia* (BA), *Berberis* (BE), *Caesalpinia* (CP), *Cassia* (CS), *Cornus* (CO), *Hamamelis* (HA), *Jasminus* (JA), *Ligustrum* (LI), *Lonicera* (LO), *Nerium* (NE), *Robinia* (RO). Columns report the number of isolated fungal species. The total number of records calculated per fungal genus is indicated as Tot. SF. The total number of records per plant genera is indicated as Tot. SP. Fungal genera are sorted by alphabetic order.

Fungi Genera	Plant Genera													
	AC	AL	BA	BE	CP	CS	CO	HA	JA	LI	LO	NE	RO	Tot SF
Acremonium		**1**	**3**											**4**
Albifimbria			**1**											**1**
Alternaria	**1**		**1**	**4**	**1**		**3**			**2**		**2**		**14**
Anguillospora				**1**										**1**
Ascochyta							**1**							**1**
Ascotricha			**2**											**2**
Aspergillus	**3**	**8**	**11**	**1**	**9**	**2**	**3**					**3**		**40**
Aureobasidium	**2**						**4**							**6**
Bacillispora				**1**										**1**
Beauveria													**1**	**1**
Bipolaris		**1**			**2**							**1**		**4**
Botryosphaeria	**1**						**2**							**3**
Botrytis			**1**				**1**							**2**
Campylospora				**1**										**1**
Cercospora				**1**										**1**
Chaetomium	**2**		**1**									**3**		**6**
Chrysosporium					**1**									**1**
Cladosporium			**4**		**1**		**5**			**1**		**3**		**14**
Clonostachys				**1**						**1**			**1**	**3**
Cochliobolus	**1**		**3**									**1**		**5**
Colletotrichum	**2**	**1**	**3**	**4**	**1**		**2**	**1**	**7**	**3**		**3**		**27**

Fungi Genera	Plant Genera													
Coprinus						1								1
Cordyceps							1							1
Corynespora			1											1
Cryptodiaporthe							1							1
Cryptodiaporthe							1							1
Curvularia		1	5		2							2		10
Cylindrocarpon												1		1
Cyrptosporiopsis							1							1
Daldinia						1								1
Diaporthe		1	1	2			2						1	7
Didymella							2							2
Diplococcium			2											2
Diplodia	1									1				2
Discula							1							1
Dothiorella	6		2											8
Drechslera												1		1
Drepanopeziza							1							1
Elsinoe							1							1
Epicoccum					1		1							2
Eupenicillium	1													1
Eutiarosporella	1													1
Exserohilum			1											2
Fusarium	1	4	4	4	4		4			2	1	4	1	29
Fusidium			1											1
Geomyces												1		1
Geotrichum			1		1					1				3
Gibberella			2											2
Glomerella			1											1
Gloniopsis													1	1
Guignardia						1				1	1			3
Heliscus				1										1
Helminthosporium					1		1							2
Hypoxylon						1								1
Khuskia			1											1
Kiflimonium			1											1
Lasiodiplodia	6	1	1		1					2		1		12
Lasmenia			2											2
Lecanicillium							1							1
Leptosphaerulina							1							1
Libertella										1				1
Lophiostoma							1							1
Microsphaeropsis				1										1
Moesziomyces	1												1	2
Myrmecridium			2											2

Fungi Genera	Plant Genera													
Myrothecium				1										3
Nectria					2									2
Nemania						1								1
Neocosmospora		1		1						1				3
Neofabraea							1							1
Neofusicoccum	6													6
Neonectria							2							2
Nigrospora			4			1	1	1				1		8
Nodulisporium			2			2								4
Oblongocollomyces	1													1
Paecilomyces		2												2
Papulospora						1								1
Paraboeremia			1											1
Paraphaeosphaeria	1			1										2
Penicillium	2	3	7		3	2	8			1		4		30
Periconia						1								1
Peroneutypa										1				1
Pestalotia	1		1											2
Pestalotiopsis			2				4			1				7
Peyronellaea	1													1
Pezicula								1						1
Phaeobotryosphaeria	1													1
Phoma	2		3				1					1		7
Phomopsis			3	1		2	3			3				12
Phyllosticta	1		1	1	1		1	1		1	1	1		9
Phytophthora							1							1
Pithomyces			1											1
Pleuroceras							1							1
Prathoda				1										1
Preussia	1													1
Psathyrella						1								1
Pseudopithomyces			1											1
Pseudothielavia												1		1
Puccinia				1										1
Pycnidiella										1				1
Rhizopus	1									1				2
Rosellinia		1												1
Sarocladium							1							1
Scedosporium			1											1
Sclerotinia							1							1
Scopulariopsis					1									1
Septoria							1							1
Simplicillium							1							1
Spegazzinia			2											2

Fungi Genera	Plant Genera													
Spencermartinsia	**1**													**1**
Sphaeria			**1**											**1**
Sporormiella			**1**											**1**
Stenella							**1**							**1**
Talaromyces			**3**		**2**		**3**							**8**
Thelioviopsis						**1**								**1**
Thelonectria							**1**							**1**
Torula												**1**		**3**
Trichoderma	**1**	**1**	**2**		**6**		**1**			**2**		**1**		**14**
Tubakia							**2**							**2**
Verticillium		**1**					**1**							**2**
Xylaria	**1**				**2**	**1**	**1**			**2**		**1**		**8**
Wickerhamomyces	**1**													**1**
Tot. SP	**51**	**27**	**94**	**29**	**42**	**19**	**78**	**4**	**7**	**29**	**3**	**37**	**6**	

The literature evidenced that several fungal endophytes live in association with the investigated plants. The most representative genera in terms of abundance of isolated species were *Aspergillus* (40 spp.), *Penicillium* (30), *Fusarium* (29), *Colletotrichum* (27), *Alternaria* (14), and *Cladosporium* (14). These genera include ubiquitous and generalist fungi as well as several plant pathogens and saprobes [40][41][42].

It is worth noting the relative homogeneity in distribution of fungi such as *Colletotrichum*, *Fusarium,* and *Alternaria* among these plant genera. In fact, *Colletotrichum* was undetected only in *Lonicera* and *Robinia*, *Fusarium* in *Caesalpinia*, and *Hamamelis, Jasminus,* and *Alternaria* in *Cassia* and *Lonicera.* Although scarcely abundant, the fungal genus *Phyllosticta* was almost reported for all selected plants except for *Albizia*, *Jasminus*, *Robinia,* and *Hamamelis.* Other endophytic fungi were detected more occasionally. Future surveys may reveal the presence of additional fungal species also from less investigated plants, such as *Robinia*, *Jasminum,* and *Lonicera*.

The presence of pathogenic or saprotrophic fungi has already been discussed by several authors [43][44]. Table 1 shows that several of the listed fungi were apparently restricted to a single plant genus or at least exhibit some preference for a particular one. Some common and ubiquitous pathogens have been recovered in more than one plant host. This is the case of *F. oxysporum* (8 host plant species belonging to 7 different genera), *A. alternata*, *A. niger*, *C. gloeosporioides* (7 host plant species), *N. oryzae* (4 host plant species), *B. dothidea*, *C. globosum, C. acutatum* (3 host plant species), *A. ochraceus*, *A. pullulans*, and *C. truncatum* (3 host plant species).

3. The Most Common Plant Pathogens

The most frequent endophytes detected from the investigated plants are cosmopolitan and ubiquitous pathogens that may cause severe yield losses. In detail, *F. oxysporum* is responsible for the wilt of vascular tissues on numerous crops that may result in plant death, even if several strains have proved to be non-pathogenic [45]. It has been isolated from 8 different plant species belonging to 7 genera, namely *A. hindsii*, *A. julibrissin*, *B. malabarica*, *B. phoenicea*, *B. aristata, C. officinalis*, *L. lucidum,* and *N. oleander.* The fungus *A. alternata* may infect over 380 host plant species causing leaf spots, rots, and blights. It includes opportunistic forms in developing field crops as well as saprophytic strains that may cause harvest and post-harvest spoilage of harvested products. One of the major concerns represented by its infection is related to the production of mycotoxins that may be introduced in the food chain [46]. In this review, *A. alternata* has been found in association with 3 genera, in 7 plant species (*B. malabarica*, *B. racemosa*, *B. poiretii*, *B. aristata*, *Cornus* sp., *L. lucidum*, and *C. pulcherrima*). The saprophytic pathogen *A. niger* is responsible for the spoilage of a wide range of fruit, vegetable, and food products. It is also the causal agent of the black rot of onion bulbs, the kernel rot of maize, and the black mold rot of cherry [47][48]. It has been found within plant tissues of *A. arabica*, *A. lebbeck*, *B. fortificata*, *B. malabarica*, *B. racemosa*, *C. pulcherrima,* and *N. oleander* (7 plant species or 4 genera). Furthermore, three different species of *Colletotrichum* have been isolated from reviewed plants. *C. gloeosporioides* has been isolated from 7 plant species (3 genera), namely *A. hindsii*, *B. racemosa*, *B. aristata*, *C. echinata*, *C. officinalis*, *C. stolonifera*, and *L. lucidum,* whereas *C. acutatum* has been found in *Cornus* spp*., Hamamelis* sp., and *H. virginiana* (3 species; 2 genera). Both *Colletotrichum* species may cause severe fruit rot mainly occurring in pre- and post-harvest [49]. Moreover, *C. truncatum,* the causal agent of anthracnose disease affecting several leguminous crops [49], has been collected from 2 plant genera, namely *A. hindsii* and *J. sambac.* Furthermore, *C. lunata*, was isolated from the tissues of 4 plant species (2 genera), including *B. malabarica, B. racemosa, B. phoenicea,* and *C. sappan*, is the causal agent of seed and seedling blight in several crops, such as rice, millet, sugarcane, and rice, and of maize leaf spot [50]. Besides, *B. dothidea* reported in association with *A. karroo*, *Cornus* sp*.,* and *C. officinalis* may cause cankers, dieback, fruit rot, and blue stain in woody plants, including *Acacia, Eucalyptus, Vitis*, and *Pistachio* [12]. Concerning the species *F. lateritium,* it has been extensively investigated as the causal agent of chlorotic leaf distortion on sweet potato (*Ipomoea batatas*) in the USA [51]. This fungus has been isolated from three different plant species and genera (*B. aristata, C. controversa,* and *L. lucidum*). Moreover, the common soil-borne fungus *G. candidum*, found in association with *B. vahlii, C. sappan,* and *L. lucidum,* is the causal agent of sour-rot of tomatoes and citrus fruits, and it is also one of the most economically important post-harvest diseases of citrus [52]. Also, *C. cladosporioides,* detected in *B. racemosa, C. echinata,* and *C. stolonifera*, is the causal agent of blossom blight in strawberries [53]. Other pathogenic fungi associated with these selected plants are less widespread and some of them are subjected to containment measures in some countries. This is the case of *N. parvum, N. oryzae, L. theobromae,* and *D. destructiva.* In particular, *N. parvum*, isolated as an endophyte in three *Acacia* species (*A. heterophylla, A. karroo,* and *A. koa*), is one of the most aggressive causal agent of Botryosphaeria dieback on the grapevine and it is known as an aggressive polyphagous pathogen attacking more than 100 plant hosts [54]. Also, *N. oryzae,* reported from *H. mollis, B. phoenicea, B. racemosa,* and *B. fortificata,* may reduce plant growth and seed quality of rice plants as well as *Brassica* spp., maize, and cotton [55]. Moreover, *L. theobromae,* found in association with six different plant species (*A. karroo, A. koa, B. racemosa, C. echinata, L. lucidum,* and *N. oleander*), is the causal agent of dieback, root rot, and blights for a wide range of plant hosts, mainly located in tropical and subtropical regions [56]. Finally, *D. desctructiva*, recovered from three different species of *Cornus*, is the causal agent of the dogwood anthracnose, a devastating disease that was firstly documented in the USA and then introduced into Europe [57].

Generally, closely related organisms, including pathogenic fungi as well as those non-pathogenic, may share similar ecological niches and may potentially interact among themselves. Their co-occurrence could be due to phylogenetic evolution or some unclear biological benefits gained [58][59]. The effects of this interaction may lead to a definition of spaces for development and survival. Nevertheless, it is widely known that non-indigenous species represent one of the greatest threats to native biodiversity [11][23][24][25]. In fact, a fungal invasion into a new ecosystem may change the native endophytic community structure, leading to the extinction of host-specialized fungi [60]. This antagonistic phenomenon is regulated by the production of antifungal compounds, mycoparasitism, or competition for space and resources [58], as well as a synergy of these interactions [59]. Biological invasions may set in motion a long-lasting cascade of effects on the plant host and associated species in unpredictable ways. Generally, the ecological importance of native species prior to the invasion may not be quantified because of the lack of information on fungal communities, especially for non-pathogenic fungal species. As a consequence of global trade and climatic or environmental changes, studies about the impact of new organisms on the ecosystem represent innovative challenges worldwide. In view of these considerations, even if fungal pathogens found in association with investigated plants are widely distributed in the EU [60][61][62][63][64][65][66][67][68], the risk posed by the introduction of potentially noxious species may be very high. Thus, our results suggest the importance of monitoring imported material to avoid the introduction of such alien species.

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