

Review

# Endophytic Fungi of Citrus Plants

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**Abstract:** Besides a diffuse research activity on drug discovery and biodiversity carried out in natural contexts, more recently, investigations concerning endophytic fungi have started considering their occurrence in crops based on the major role that these microorganisms have been recognized to play in plant protection and growth promotion. Fruit growing is particularly involved in this new wave, by reason that the pluriannual crop cycle likely implies a higher impact of these symbiotic interactions. Aspects concerning occurrence and effects of endophytic fungi associated with citrus species are revised in the present paper.

**Keywords:** *Citrus* spp.; endophytes; antagonism; defensive mutualism; plant growth promotion; bioactive compounds

## 1. Introduction

Despite the early pioneering observations dating back to the nineteenth century [1], a settled prejudice that pathogens basically were the only microorganisms able to colonize living plant tissues has long delayed the awareness that endophytic fungi are constantly associated to plants, and remarkably influence their ecological fitness. Overcoming an apparent vagueness of the concept of ‘endophyte’, scientists working in the field have agreed on the opportunity of delimiting what belongs to this functional category. Thus, a series of definitions have been enunciated which are all based on the condition of not causing any immediate overt negative effect to the host [2].

Besides being prompted by the general theoretical intent that all components of biodiversity from natural contexts ought to be exploited for the benefit of humanity, investigations on the endophytic microbiota, or endosphere [3], have also been undertaken with reference to crops. In this respect, it can be said that endophytes are even more relevant in orchards, where the time factor confers higher impact to the establishment of an equilibrium among the species which are part of the tree biocoenosis, and to its possible disruption. Hence, all sorts of contributions have recently been proliferating in the literature, to such an extent that an organization of the available information is now appropriate in order to support the scientific community in achieving further progress. In view of this perspective, the present paper offers a review of the state-of-the-art of research concerning occurrence and effects of endophytic fungi associated with citrus species.

## 2. Endophytic Occurrence of Citrus Pathogens

The agent of citrus black spot (CBS) *Phyllosticta citricarpa*, also known under the teleomorph name *Guignardia citricarpa* (Dothideomycetes, Botryosphaeriaceae), is one of the most noxious pests of these crops in subtropical regions, and it is subject to phytosanitary restrictions by the European Union and the United States. The employment of biomolecular methods has provided substantial support to the distinction between pathogenic isolates, typically slow-growing in axenic cultures and producing a yellow halo on oatmeal agar, and non-pathogenic isolates, which are morphologically

similar but fast-growing, and producing conidia embedded within a thicker mucoid sheath [4–8]. The latter, characterized as a different species (*Phyllosticta capitalensis*), are known to be ubiquitous as endophytes in woody plants, having been reported from at least 70 botanical families [6,9,10]. *Guignardia endophyllicola*, treated as a separate species in a work also emphasizing its widespread endophytic occurrence [11], is at present recognized as a synonym. Differences between the two sister species also concern their metagenetic cycle. In fact, it has been ascertained that *P. citricarpa* is heterothallic, while *P. capitalensis* is homothallic [8]. This consolidated taxonomic distinction supports the exclusion from quarantine measures of plant material harbouring *P. capitalensis*. To this purpose, several rapid PCR assays have been developed [12–20]. The applicative use of these assays has enabled to exclude the presence of the pathogen in New Zealand, unlike what was previously assumed [21], and has supported the hypothesis of the possible endophytic occurrence of *P. citricarpa* in asymptomatic *Citrus* spp., as pointed out by several investigations (Table 1). Moreover, the two species have been clearly differentiated on account of their enzymatic profiles, with a higher expression of amylases, endoglucanases, and pectinases in *P. citricarpa*, suggesting a likely involvement of these enzymes in the pathogenic aptitude of the CBS agent [22]. Differences in terms of pathogenesis-related proteins have been confirmed after the genome sequencing of the two species, disclosing a higher number of coding sequences in *P. citricarpa* (15,206 versus 14,797). Such a difference has been interpreted considering the presence of growth and developmental genes involved in the expression of pathogenicity [23].

The issue of detection of contaminated material imported from areas where the pathogen is endemic has also prompted investigations concerning the assortment of *Phyllosticta* spp. able to colonize citrus plants in either symptomatic or latent courses. Several revisions have been published [17,24], and novel species characterized, which consistently enlarge the citrus-associated consortium within this widespread genus. Particularly, the pathogenic *P. citriasiatica* from south-east Asia [25], *P. citrichinaensis* from China [26], *P. citrimaxima* from Thailand [24], and *P. paracitricarpa* from Greece [27], and the non-pathogenic endophytic *P. citribraziliensis* from Brazil [28] and *P. paracapitalensis* from New Zealand, Italy, and Spain [27]. The isolation by the latter research group of *P. citricarpa* from specimen collected in citrus groves in Italy, Malta, and Portugal, following analogue findings in Florida [19,29], is expected to provide impulse for a more thorough assessment of distribution and pathogenicity of this species [30]. A very recent investigation carried out in Australia on several *Citrus* spp. and growing conditions, has disclosed *P. paracapitalensis* to be even more widespread than *P. capitalensis*. Strains of both species were confirmed to be non-pathogenic on fruits under field conditions, and displayed antagonistic effects against the CBS agent, introducing their possible exploitation in the integrated management of this disease [31]. In this respect, it has been speculated that, rather than depending on intrinsic genetic factors, resistance to CBS by *C. latifolia* could be due to its systematic colonization by *P. capitalensis*, as disclosed by a dedicated investigation carried out in Brazil [32].

*Colletotrichum* (Sordariomycetes, Glomerellaceae) is another important ascomycete genus in course of coherent taxonomic revision. Besides *Colletotrichum gloeosporioides*, the agent of citrus anthracnose, it includes many species known for their endophytic aptitude. A recent investigation carried out in China on several *Citrus* spp. has shown a high proportion of endophytic strains to belong to *C. gloeosporioides sensu stricto*, calling for further investigations concerning the asymptomatic occurrence of this pathogen in citrus orchards. Additional identified species are *Colletotrichum fructicola* from *Citrus reticulata* cv. Nanfengmiju and *Citrus japonica* (= *Fortunella margarita*), and *Colletotrichum karstii* [33]. A similar widespread occurrence of *C. gloeosporioides* has been more recently confirmed in Brazil, where just one out of 188 isolates was found to be able to induce post-bloom fruit drop. This syndrome is more frequently associated to the species *Colletotrichum abscissum*, which, however, does not display an endophytic habit [34]. Endophytic *C. gloeosporioides* were also previously reported from *Citrus limon* in Argentina [35] and Cameroon [36].

One more meaningful example of endophytic fungus converting to pathogenic when plants are exposed to stress factors is represented by another member of the Botryosphaeriaceae, *Lasiodiplodia theobromae*. Characterized by a widespread endophytic occurrence [37,38], this species has been

reported to exacerbate pre-harvest fruit drop and post-harvest fruit decay in plants of *Citrus sinensis* hit by the huanglongbing syndrome [39].

A quite intricate case deserving further investigations with reference to the epidemiological impact by endophytic strains is represented by members of the genus *Diaporthe* (Sordariomycetes, Diaporthaceae), also known under the anamorph name *Phomopsis* [40,41], which are widespread in different ecological contexts [41,42]. Besides the longtime known *D. citri*, more species in this genus have been recently identified as the causal agents of melanose, stem-end rot, and gummosis on *Citrus* spp., particularly, *D. citriasiana* and *D. citrichinensis* in China [43], and *D. limonicola*, *D. melitensis*, *D. baccae*, *D. foeniculina*, and *D. novem* in Europe [44]. Even more species have been reported for their endophytic occurrence as a result of a phylogenetic reassessment carried out in China, with eight known (*D. arecae* species complex, *D. citri*, *D. citriasiana*, *D. citrichinensis*, *D. endophytica*, *D. eres*, *D. hongkongensis*, and *D. sojiae*) and seven new species (*D. biconispora*, *D. biguttulata*, *D. discoidispora*, *D. multiguttulata*, *D. ovalispora*, *D. subclavata*, and *D. unshiuensis*) [45].

**Table 1.** Endophytic fungi reported from *Citrus* species.

Endophyte <sup>1</sup>	Plant Species	Country	Reference
	<i>C. limon</i> , <i>C. tangelo</i>	Florida	[46]
<i>Alternaria alternata</i>	<i>Citrus</i> spp.	Japan	[47]
	<i>C. limon</i>	Argentina	[35]
<i>Alternaria brassicicola</i>	<i>C. reticulata</i>	Iran	[48]
	<i>C. reticulata</i>	Iran	[48]
<i>Alternaria carthami</i>	<i>C. reticulata</i>	Iran	[48]
<i>Alternaria citri</i>	<i>C. sinensis</i>	Iran	[49]
<i>Alternaria infectoria</i>	<i>C. sinensis</i>	Iran	[49]
<i>Alternaria rosae</i>	<i>C. sinensis</i>	Iran	[49]
<i>Alternaria</i> sp.	<i>C. kotokan</i>	Taiwan	[52]
	<i>C. sinensis</i>	Iran	[49]
<i>Annulohyphoxylon stygium</i>	<i>C. sinensis</i>	Iran	[49]
<i>Arthrinium</i> sp.	<i>C. japonica</i>	Taiwan	[52]
<i>Ascochyta medicaginicola</i>	<i>C. reticulata</i>	Iran	[48]
<i>Aspergillus nidulans</i>	<i>C. sinensis</i>	Iran	[49]
<i>Aspergillus niger</i>	<i>C. reticulata</i>	Iran	[48]
<i>Aspergillus pallidofulvus</i>	<i>C. reticulata</i>	Iran	[48]
<i>Aspergillus terreus</i>	<i>C. sinensis</i>	Iran	[49]
<i>Aureobasidium iranianum</i>	<i>C. reticulata</i>	Iran	[48]
<i>Aureobasidium melanogenum</i>	<i>C. reticulata</i>	Iran	[48]
	<i>C. sinensis</i>	Brazil	[53]
<i>Aureobasidium pullulans</i>	<i>C. japonica</i>	Uruguay	[54]
	<i>C. reticulata</i>	Iran	[48]
<i>Beauveria bassiana</i>	<i>C. limon</i>	China	[55]
<i>Biscogniauxia mediterranea</i>	<i>C. sinensis</i>	Iran	[49]
<i>Biscogniauxia nummularia</i>	<i>C. sinensis</i>	Iran	[49]
<i>Bjerkandera adusta</i>	<i>C. sinensis</i>	Iran	[49]
<i>Botryosphaeria</i> sp.	<i>C. aurantium</i>	Taiwan	[52]
<i>Camarosporium</i> sp.	<i>C. aurantium</i> , <i>C. medica</i> var. <i>sarcodactylis</i>	Taiwan	[52]
<i>Candida parapsilosis</i>	<i>C. sinensis</i>	Brazil	[53]
<i>Cercospora</i> sp.	<i>C. limon</i>	Cameroon	[36]
	<i>C. sinensis</i>	Iran	[49]
<i>Chaetomium globosum</i>	<i>C. sinensis</i>	Iran	[49]
<i>Chaetomium</i> sp.	<i>C. sinensis</i>	Taiwan	[52]
<i>Cladosporium cladosporioides</i>	<i>C. reticulata</i>	Iran	[48]
<i>Cladosporium</i> sp.	<i>C. limon</i> , <i>C. reshni</i> , <i>C. sinensis</i> , <i>C. sunki</i> , <i>C. trifoliata</i> , <i>C. volkameriana</i>	Brazil	[56]
	<i>C. reticulata</i>	Iran	[48]
<i>Cladosporium xanthochromaticum</i>	<i>C. limon</i>	Cameroon	[36]
<i>Colletotrichum boninense</i>	<i>C. sinensis</i>	Iran	[49]

Table 1. Cont.

Endophyte <sup>1</sup>	Plant Species	Country	Reference
<i>Colletotrichum fructicola</i>	<i>C. japonica</i> , <i>C. reticulata</i>	China	[43]
	<i>C. limon</i> , <i>C. reshni</i> , <i>C. sinensis</i> , <i>C. sunki</i> , <i>C. trifoliata</i> , <i>C. volkameriana</i>	Brazil	[56]
<i>Colletotrichum gloeosporioides</i>	<i>C. limon</i>	Argentina	[35]
	<i>C. grandis</i> , <i>C. reticulata</i> , <i>C. sinensis</i> , <i>C. unshiu</i>	Cameroon	[36]
<i>Colletotrichum karstii</i>	<i>C. sinensis</i>	China	[43]
	<i>C. grandis</i> , <i>C. limon</i>	Iran	[49]
<i>Colletotrichum sp.</i>	<i>C. aurantium</i> , <i>C. medica</i> var. <i>sarcodactylis</i> , <i>C. sinensis</i>	China	[43]
	<i>C. deliciosa</i> , <i>C. reticulata</i>	Taiwan	[52]
<i>Coprinellus radians</i>	<i>C. sinensis</i>	Brazil	[57]
<i>Coprinopsis</i> sp.	<i>C. aurantifolia</i>	India	[58]
<i>Cryptococcus flavescens</i>	<i>C. sinensis</i>	Iran	[49]
<i>Cryptococcus laurentii</i>	<i>C. medica</i>	Taiwan	[52]
<i>Cyanodermella</i> sp.	<i>C. sinensis</i>	Brazil	[53]
<i>Diaporthe arecae</i> s.c. <sup>2</sup>	<i>C. medica</i> var. <i>sarcodactylis</i> , <i>Citrus</i> sp.	Brazil	[53]
	<i>C. grandis</i> , <i>C. limon</i> , <i>C. reticulata</i> , <i>C. sinensis</i> , <i>Citrus</i> sp., <i>C. unshiu</i>	Taiwan	[52]
<i>Diaporthe biconispora</i> <sup>2,*</sup>	<i>C. grandis</i> , <i>C. japonica</i> , <i>C. sinensis</i>	China	[45]
<i>Diaporthe biguttulata</i> <sup>2,*</sup>	<i>C. limon</i>	China	[45]
<i>Diaporthe citri</i> <sup>2</sup>	<i>C. reticulata</i> , <i>C. unshiu</i>	China	[43,45]
<i>Diaporthe citriasiana</i> <sup>2</sup>	<i>C. unshiu</i>	China	[43]
<i>Diaporthe citrichinensis</i> <sup>2</sup>	<i>C. grandis</i> , <i>C. japonica</i>	China	[45]
<i>Diaporthe discoidispora</i> <sup>2,*</sup>	<i>C. sinensis</i> , <i>C. unshiu</i>	China	[45]
<i>Diaporthe endophytica</i> <sup>2</sup>	<i>C. limon</i>	China	[45]
<i>Diaporthe eres</i> <sup>2</sup>	<i>C. japonica</i> , <i>Citrus</i> sp., <i>C. unshiu</i>	China	[45]
<i>Diaporthe eucalyptorum</i> <sup>2</sup>	<i>C. limon</i>	Cameroon	[36]
<i>Diaporthe foeniculina</i> <sup>2</sup>	<i>C. sinensis</i>	Iran	[49]
<i>Diaporthe hongkongensis</i> <sup>2</sup>	<i>C. grandis</i> , <i>C. reticulata</i> , <i>C. sinensis</i> , <i>C. unshiu</i>	China	[45]
<i>Diaporthe multiguttulata</i> <sup>2,*</sup>	<i>C. grandis</i>	China	[45]
<i>Diaporthe ovalispora</i> <sup>2,*</sup>	<i>C. limon</i>	China	[45]
<i>Diaporthe phaseolorum</i> <sup>2</sup>	<i>C. limon</i>	Cameroon	[36]
<i>Diaporthe sojae</i> <sup>2</sup>	<i>C. limon</i> , <i>C. reticulata</i> , <i>C. unshiu</i>	China	[45]
<i>Diaporthe</i> sp. <sup>2</sup>	<i>C. limon</i>	Cameroon	[36]
	<i>C. aurantium</i> , <i>C. medica</i> , <i>C. sinensis</i>	Taiwan	[52]
<i>Diaporthe unshiuensis</i> <sup>2,*</sup>	<i>C. japonica</i>	China	[45]
	<i>C. reticulata</i>	Iran	[48]
<i>Didymella microchlamydospora</i>	<i>C. japonica</i>	China	[45]
<i>Discostroma</i> sp.	<i>C. reticulata</i>	Iran	[48]
<i>Epicoccum nigrum</i>	<i>C. medica</i>	Taiwan	[52]
<i>Eutypella</i> sp.	<i>C. sinensis</i>	Iran	[49]
<i>Fusarium culmorum</i>	<i>C. medica</i> var. <i>sarcodactylis</i>	Taiwan	[52]
<i>Fusarium incarnatum</i>	<i>C. sinensis</i>	Iran	[49]
<i>Fusarium oxysporum</i>	<i>C. sinensis</i>	Iran	[49]
<i>Fusarium proliferatum</i>	<i>C. reticulata</i>	Iran	[48]
<i>Fusarium sarcochroum</i>	<i>C. sinensis</i>	Iran	[49]
<i>Fusarium</i> sp.	<i>C. limon</i> , <i>C. reticulata</i>	Italy, Spain	[50]
	<i>C. sinensis</i>	Taiwan	[52]
<i>Hanseniopsis opuntiae</i>	<i>C. reticulata</i>	Iran	[48]
<i>Hypholoma fasciculare</i>	<i>C. reticulata</i>	China	[59]
<i>Hypoxyton investiens</i>	<i>C. sinensis</i>	Iran	[49]
<i>Lasiodiplodia theobromae</i>	<i>C. sinensis</i>	Iran	[49]
<i>Lasmenia</i> sp.	<i>C. sinensis</i>	China	[39]
<i>Meira geulakonigae</i>	<i>C. medica</i> var. <i>sarcodactylis</i>	Taiwan	[52]
<i>Meyerozyma caribbica</i>	<i>C. paradisi</i>	Israel	[60]
<i>Meyerozyma guilliermondii</i>	<i>C. reticulata</i>	Iran	[48]
	<i>C. sinensis</i>	Brazil	[53]
<i>Muscador</i> sp.	<i>C. reticulata</i>	China	[58]
<i>Mycocleptodiscus</i> sp.	<i>C. sinensis</i>	Brazil	[61]
<i>Mycosphaerella</i> sp.	<i>C. aurantium</i>	Taiwan	[52]
<i>Myrothecium</i> sp.	<i>C. limon</i>	Cameroon	[36]
<i>Neocosmospora solani</i>	<i>C. reticulata</i>	Iran	[48]

Table 1. Cont.

Endophyte <sup>1</sup>	Plant Species	Country	Reference
<i>Neosectophoma</i> sp.	<i>C. reticulata</i>	Iran	[48]
<i>Nigrospora oryzae</i>	<i>C. sinensis</i>	Iran	[49]
<i>Nigrospora sphaerica</i>	<i>C. limon</i>	Argentina	[35]
<i>Nodulisporium</i> sp.	<i>C. limon</i>	Argentina	[35]
<i>Passalora loranhi</i>	<i>C. limon</i>	Cameroon	[36]
<i>Penicillium citrinum</i>	<i>C. reticulata</i>	Iran	[48]
<i>Pestalotiopsis mangiferae</i>	<i>C. limon</i>	Cameroon	[36]
<i>Pestalotiopsis microspora</i>	<i>C. limon</i>	Cameroon	[36]
<i>Pestalotiopsis</i> sp.	<i>C. limon</i>	Cameroon	[36]
<i>Phaeoacremonium parasiticum</i>	<i>C. reticulata</i>	Iran	[48]
<i>Phialophora</i> sp.	<i>C. sinensis</i>	Brazil	[53]
<i>Phoma</i> sp.	<i>C. limon</i>	Cameroon	[36]
	<i>Citrus</i> spp.	South Africa	[4]
	<i>C. deliciosa</i> , <i>C. reticulata</i>	Brazil	[57]
	<i>C. aurantium</i> , <i>C. natsudaikai</i> , <i>C. trifoliata</i>	Japan	[11]
	<i>C. aurantium</i>	Brazil	[62]
	<i>C. latifolia</i>	Brazil	[17]
<i>Phyllosticta capitalensis</i> <sup>2</sup>	<i>C. limonia</i> , <i>C. sinensis</i> , <i>Citrus</i> sp.	Brazil	[28]
	<i>C. aurantium</i> , <i>C. australasica</i>	Australia	[63]
	<i>C. limon</i>	Cameroon	[36]
		Italy, Malta, Spain Greece, Portugal	[27]
	<i>C. aurantifolia</i>	Italy	
	<i>C. sinensis</i>	Iran	[49]
<i>Phyllosticta citribraziliensis</i> <sup>2,*</sup>	<i>Citrus</i> sp.	Brazil	[28]
	<i>Citrus</i> sp.	South Africa	[64]
	<i>C. reshmi</i> , <i>C. sinensis</i> , <i>C. sunki</i> , <i>C. trifoliata</i> , <i>C. volkameriana</i>	Brazil	[56]
<i>Phyllosticta citricarpa</i> <sup>2</sup>	<i>C. deliciosa</i> , <i>C. reticulata</i>	Brazil	[65]
	<i>C. limon</i>	Argentina	[35]
	<i>C. latifolia</i>	Brazil	[17]
	<i>C. sinensis</i>	Florida	[29]
	<i>C. aurantifolia</i>	New Zealand	
<i>Phyllosticta paracapitalensis</i> <sup>2,*</sup>	<i>C. floridana</i>	Italy	[27]
	<i>C. limon</i>	Spain	
	<i>C. aurantium</i> , <i>C. australasica</i> , <i>C. hystrix</i> , <i>C. japonica</i> , <i>C. maxima</i> , <i>C. reticulata</i> , <i>C. wintersii</i>	Australia	[31]
<i>Phyllosticta</i> sp. <sup>2</sup>	<i>C. medica</i> var. <i>sarcodactylis</i>	Taiwan	[52]
<i>Physoderma citri</i>	<i>Citrus</i> spp.	Florida	[51]
<i>Pichia kluyveri</i>	<i>C. reticulata</i>	China	[59]
<i>Pseudocercospora</i> sp.	<i>C. japonica</i>	Taiwan	[52]
<i>Pseudoestalotiopsis theae</i>	<i>C. limon</i>	Cameroon	[36]
<i>Pseudozyma flocculosa</i>	<i>C. reticulata</i>	Iran	[48]
<i>Rhodotorula dairenensis</i>	<i>C. sinensis</i>	Brazil	[53]
<i>Rhodotorula mucilaginoso</i>	<i>C. sinensis</i>	Brazil	[53]
<i>Rosellinia</i> sp.	<i>C. sinensis</i>	Iran	[49]
<i>Sarocladium subulatum</i>	<i>C. sinensis</i>	Iran	[49]
<i>Scedosporium apiospermum</i>	<i>C. reticulata</i>	Iran	[48]
<i>Sordaria fimicola</i>	<i>C. sinensis</i>	Iran	[49]
<i>Sporobolomyces</i> sp.	<i>C. sinensis</i>	Brazil	[53]
<i>Sporormiella minima</i>	<i>C. limon</i>	Argentina	[35]
	<i>C. sinensis</i>	Iran	[49]
<i>Stemphylium</i> sp.	<i>C. aurantium</i> , <i>C. japonica</i>	Taiwan	[52]
<i>Stenella</i> sp.	<i>C. limon</i>	Cameroon	[36]
<i>Talaromyces purpurogenus</i>	<i>C. reticulata</i>	Iran	[48]
<i>Talaromyces trachyspermus</i>	<i>C. reticulata</i>	Iran	[48]
<i>Xylaria cubensis</i>	<i>C. sinensis</i>	Iran	[49]
	<i>C. limon</i>	Cameroon	[36]
<i>Xylaria</i> sp.	<i>C. japonica</i>	Taiwan	[52]
<i>Zasmidium</i> sp.	<i>C. limon</i>	Cameroon	[36]

<sup>1</sup> Species are reported according to the latest accepted name, which might not correspond to the one used in the corresponding reference. <sup>2</sup> Conforming to the principle 'one fungus—one name' [66], the older genus names *Diaporthe* and *Phyllosticta* have been considered to deserve priority over *Phomopsis* and *Guignardia*, respectively.

\* Novel species described for the first time from this plant source.

Endophytic occurrence has also been reported for other citrus pathogens, such as the leaf-spot agents *Alternaria alternata* [35,46–48] and *Alternaria citri* [49], *Fusarium oxysporum* [48], and *Fusarium sarcocroum*, known as a possible agent of dieback of twigs on mandarin and lemon [50]. The latter study also introduces new *Fusarium* spp. (*F. citricola*, *F. salinense*, *F. siculi*), causing cankers on several citrus species. Considering that pathogenic *Fusaria* often present an early latent stage, this finding claims for further assessments concerning their possible endophytic occurrence. Finally, it is worth mentioning *Physotheria citri*, a species ascribed to the phylum Blastocladiomycota reported to cause vessel occlusion, but also found in asymptomatic plants of several *Citrus* spp. [51].

### 3. Other Endophytic Fungi and Their Interactions with Pests and Pathogens of *Citrus*

Besides the above reports, essentially dedicated to pathogenic species/genera upon the aim to assess the epidemiological impact of latent endophytic stages, additional data have been recorded on the overall species assemblage in a few contexts (Table 1). A study carried out on *C. limon* in Cameroon [36] pointed out that yellowing of leaves affects foliar endophytic communities, and that interactions among endophytes may be a factor driving the yellowing process. In fact, yellow leaves presented a less varied species assortment dominated by *C. gloeosporioides* in the absence of species belonging to the Mycosphaerellaceae, otherwise common in healthy leaves. In vitro observations in dual cultures showed that the latter were inhibited and overgrown by *C. gloeosporioides*, even if capable to revert this inhibitory effect when pre-inoculated, which was interpreted as deriving from production of fungitoxic metabolites. This study also demonstrated a low occurrence of species in the Xylariaceae, which are usually quite widespread as tree endophytes [67,68].

The endophytic occurrence of a few yeast species was documented in an investigation carried out on *C. sinensis* in Brazil [53]. By means of scanning electron microscopy, it was observed that these microorganisms are mostly localized around stomata and in xylem vessels. Isolates of the species *Rhodotorula mucilaginosa*, *Meyerozyma (Pichia) guilliermondii*, and *Cryptococcus flavescens* were inoculated in healthy plants, and re-isolated, without causing any kind of disease symptoms. Quite interestingly, the authors noted that *M. guilliermondii* primarily occurred in plants colonized by *Xylella fastidiosa*, the causal agent of citrus variegated chlorosis (CVC), and that the bacterium could thrive on a supernatant separated from cultures of a strain of this species. This finding represents an indication that the presence of the yeast could stimulate the pathogen and could be responsible for more severe disease symptoms. More recently, strains of *M. guilliermondii* have been recovered, along with strains of *Hanseniaspora opuntiae* and *Pichia kluyveri*, from tangerine peel in China. However, it is questionable if this record can actually concern endophytic occurrence considering that authors refer that fruits were purchased on the market rather than being directly collected in the field [59].

Indeed, interactions between endophytic bacteria and fungi are complex, and the assortment of strains which can be recovered is largely influenced by the antagonistic interactions as mediated by the production of antibiotics. In this respect, strains of *P. citricarpa* isolated from *Citrus* spp. in Brazil were found to possess inhibitory properties toward several endophytic *Bacillus* spp. from the same source, while a stimulatory effect was assessed towards the gram-negative *Pantoea agglomerans*, which can be taken as an indication of the opportunity to investigate possible interference with the development of *X. fastidiosa* [56].

Antagonistic properties by an isolate of *Muscodora* sp. from *C. sinensis* were reported against *P. citricarpa* as deriving from the production of volatile organic compounds (VOCs) [61]. Actually, such properties are known for endophytic isolates of *Muscodora* and other genera of xylariaceous fungi, such as *Hypoxylon (=Nodulisporium)* and *Xylaria*, reported from many plant species [69] and also occurring in citrus plants [35,36,49,52].

Endophytic strains belonging to two species of *Diaporthe*, *D. terebinthifolii* and the already-mentioned *D. endophytica*, displayed inhibitory properties against *P. citricarpa* in vitro and on detached fruits. Moreover, their transformants expressing the fluorescent protein DsRed proved to be able to actively colonize citrus seedlings, and to remain viable in the plant tissues for one year at least. These evidences support their

possible use in the biocontrol of this pathogen [70]. Antifungal properties have also been reported for a strain of another fungus belonging to the Diaporthales (*Lasmenia* sp.), which was recovered from *C. medica* var. *sarcodactylis* [52].

Rather than just concerning agents of cryptogamic diseases, protective effects by endophytic fungi may pertain several kinds of pests [71,72]. Actually, data available in the literature concerning citrus plants are limited but encourage further assessments. For instance, a ustilaginomycetous yeast endophytic in grapefruit (*Citrus paradisi*), *Meira geulakonigae*, was found to be able to reduce populations of the citrus rust mite (*Phyllocoptruta oleivora*) [60]. More recently, two strains of *Beauveria bassiana* were inoculated in seedlings of *C. limon* through foliar sprays and proved to be able to colonize the plants endophytically. Besides increasing plant growth, they caused 10%–15% mortality on adults of the Asian citrus psyllid (*Diaphorina citri*), and the females feeding on the treated plants laid significantly fewer eggs [55]. It is not unlikely that more evidence in this respect can be gathered from targeted investigations concerning naturally occurring endophytes, considering that protective effects have been documented for endophytic strains of *F. oxysporum* against aphids [73] and nematodes [74].

As a general ecological trait, endophytic fungi seem to be absent in seeds of citrus species [65]. This is to be taken as an indication that these microorganisms are not adapted to a vertical spread, and most likely colonize citrus plants coming from the surrounding environment.

#### 4. Biotechnological Implications

The involvement of endophytic fungi in a tripartite relationship with the host plant and its pests and pathogens highlights their basic role in establishing an equilibrium in such a fragile biocoenosis. Indeed, a major biotechnological application of endophytic strains consists in the exploitation of their aptitude to defensive mutualism.

The endophytic habit is conducive for interactions with other microorganisms sharing the same micro-environment. There is strong evidence that these interactions entangle the genetic level, and that interspecific transfer of gene pools regularly occurs. Probably, the best example in this respect is represented by genes encoding for the synthesis of polyketide secondary metabolites, which are usually grouped in clusters and are influenced in their expression by several external factors [75,76]. Horizontal gene transfer from other endophytic microorganisms may eventually explain the ability by a strain of *P. citricarpa* [77] to produce the blockbuster drug taxol, first extracted from *Taxus* spp. and afterwards as a secondary metabolite of a high number of endophytic fungi [69,78].

*P. citricarpa* has been further characterized with reference to production of secondary metabolites. Particularly, it has been reported to produce the new dioxolanone phenguignardic acid butyl ester, along with four previously reported compounds: phenguignardic acid methyl ester, peniisocoumarin G, protocatechuic acid, and tyrosol [79]. *Phyllosticta* spp. have been reported to have a similar metabolomic profile, including the dioxolanone phytochemicals which are regarded as potential virulence factors. However, one of these products, guignardic acid, has also been reported from *P. capitalensis* [80]. Biosynthetic abilities by endophytic strains of the latter species also refer to meroterpenes, such as compounds in the guignardone series [81–84] and the manginoids [85]. Besides a likely implication in the relationships with other citrus-associated microbial species, the bioactive properties of the dioxolanones and the related meroterpene compounds deserve to be further investigated in view of possible pharmaceutical exploitation [79,86].

Protocatechuic acid was again reported from an unidentified fungal strain recovered from leaves of *Citrus jambhiri*, along with indole-3-acetic acid (IAA) and acropyrone [87]. The latter compound was shown to possess antibiotic properties against *Staphylococcus aureus*, while the finding of IAA is in line with the many reports of plant hormones produced by endophytic fungi [69], which at least in part unfold the growth-promoting effects exerted on their hosts [88,89]. Production of IAA was also reported from strains of the yeasts *Hanseniaspora opuntiae* and *Meyerozyma guilliermondii* from *Citrus reticulata*, which were able to induce growth-promoting effects on seedlings of *Triticum aestivum* [59].

The above-mentioned VOCs reported from an endophytic strain of *Muscodor* sp. from *C. sinensis* include several sesquiterpenes, namely azulene, cis/trans- $\alpha$ -bergamotene, cedrene, (Z)- $\beta$ -farnesene, farnesene epoxide,  $\alpha$ -himachalene,  $\alpha$ -longipinene, thujopsene, 2,4,6-trimethyl-1,3,6-heptatriene, 2-methyl-5,7-dimethylene-1-8-nonadiene, and cis-Z-bisabolene epoxide [61]. Mixtures of these compounds have a possible biotechnological application for the mycofumigation of fruits, proposed for the control of CBS and various post-harvest pathogens [90–92]. Concerning VOCs, another possible investigational subject consists in assessing if any endophyte of citrus plants is able to produce compounds occurring in the typical aroma spread by flowers and fruits of these plants, which are exploited by the pharmaceutical and the perfume industries. In this respect, the production of bergapten, a psoralen compound known from bergamot (*Citrus bergamia*), has already been pointed out by endophytic strains of *Penicillium* sp. [93] and *L. theobromae* [94]. Although these findings concern plants other than citrus, it is worth considering that these fungi are also reported as citrus endophytes (Table 1).

Antimicrobial properties of fungi do not just depend on the production of bioactive compounds. In fact, a strain of *P. capitalensis* (Bios PTK 4) recovered from an unidentified citrus plant was found to be able to synthesize silver nanoparticles extracellularly. These nanoparticles, which were spherical, 5–30 nm in size, well-dispersed, and extremely stable, have been characterized for their antibacterial and antifungal properties [95].

## 5. Conclusions

Revision of literature in the field shows that a major part of the research activity carried out on endophytic fungi of citrus plants consists in investigations on the occurrence of pathogens, and their discrimination from other ecologically associated taxa. Such a limited approach has, anyway, turned to be useful to disclose an epidemiological relevance of these microorganisms, as related to a modulatory role in the spread of citrus diseases. On that account, possible interactions in conducive contexts with other important pathogens, such as the agent of mal secco *Phoma tracheiphila* and species of *Phytophthora* causing foot and root rot, should be attentively considered. Even when there is no apparent direct interaction with disease agents, such as in the cases of CVC incited by *X. fastidiosa*, tristeza, and other viroses, the possible effect by endophytic fungi in stimulating plant defense reaction, or, more in general, to act as plant disease modifiers [96], should not be disregarded. In this respect, data concerning occasional isolations might well disclose some relevance. Unfortunately, description of the endophytic assemblages in several papers is often approximate or incomplete, such as in a mentioned survey concerning sweet orange (*C. sinensis*), where just a single strain was characterized out of a sample of over 400 endophytes [61]. It is to be recommended that future investigations in the field be more circumstantial in their approach to describe this component of biodiversity, in the aim of increasing opportunities for its biotechnological exploitation.

Encouraging examples in this direction are represented by two very recent publications from Iran [48,49]. Indeed, the focus on endophytic fungi is gradually evolving from a basically descriptive phase to the analysis of factors influencing the structure and composition of microbiomes, in view of their manipulation for increasing plant protection and productivity. A better comprehension of the already introduced genetic interactions among members of the associated biota and the host tree is crucial for the success of any practical application of endophytic fungi in sustainable agriculture [97]. Moreover, the observed effects of the host genotype [98,99] could be adequately considered in breeding programs, in the aim to select suitable recipient genotypes for fungal inoculants.

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