Symbiosis (2021) 85:31–46
https://doi.org/10.1007/s13199-021-00786-0

Plant-microbe features of *Dendrobium fimbriatum* (Orchidaceae) fungal community

Quentin Favre-Godal[1,2] · Paul Schwob[2] · Nicole Lecoultre[3] · Valérie Hofstetter[3] · Lorène Gourguillon[1] · Ludivine Riffault-Valois[2] · Sonia Lordel-Madeleine[2] · Katia Gindro[3] · Patrick Choisy[1]

Received: 4 January 2021 / Accepted: 14 June 2021 / Published online: 23 June 2021

Abstract
To document in more details our knowledge of the fungal community associated to the Orchidaceae family, 25 cultivable fungal species were isolated from greenhouse apparently healthy *Dendrobium fimbriatum* var. *oculatum* Hook. adult plants. These fungi, were identified based on a molecular approach, belonging predominantly to *Fusarium*, *Trichoderma*, *Colletotrichum*, *Curvularia* and *Didymella* genera. To characterize their impact on plant growth and/or plant defense, their ability to produce different metabolites (phytohormones, siderophores) and hydrolytic enzymes, as well as their anti-phytopathogenic activity against *Botrytis cinerea*, *Colletotrichum gloeosporioides* and *Fusarium oxysporum,* were evaluated ex situ. Based on these criteria, three fungi, initially isolated from the roots, *Fusarium* sp., *Trichoderma yunnanense*, and *Curvularia* sp., showed a high potential to act on plant growth. Our results also show that an important number of isolates of the fungal community were able to produce phytohormones, siderophores, and had a similar hydrolytic enzyme production profile. Finally, these fungal abilities, studied ex situ, were analyzed to determine whether they can be used to classify the fungal partners as beneficial or detrimental to their host.

Keywords Orchidaceae · Fungal community · Interaction · *Dendrobium fimbriatum* · Plant growth promotion · Beneficial

1 Introduction

1.1 Dendrobium and microorganism's relationships

Dendrobium is widely distributed in Asia. It is the second largest genus after *Bulbophyllum* in the Orchidaceae family with over 1500 species (Givnish et al. 2015). In *Dendrobium* genus, at least 16.4% of the species are endangered. This number is an estimation as conservation data is still missing for 28.8% of the species (International Union for Conservation of Nature red list). This genus contains the highest number of traditional vascular medicinal epiphytes (Nugraha et al. 2020). Indeed, *Dendrobium* species have been used as first-rate herbs and prized folk medicine in Asia for a thousand years (Ng et al. 2012; Cakova et al. 2017).

It was reviewed recently that 131 compounds from *Dendrobium* plants have been reported to possess anti-inflammatory, antimicrobial, antioxidant, antiaging, anti-psoriasis, and tyrosinase-inhibitory activities, highlighting this genera as an important resource for the development of new drugs and cosmetics (Wang 2021).

Dendrobium fimbriatum var. *ocultatum,* belonging to the so called "Fengdou Shihu" in traditional Chinese medicine (TCM), is found in Southeast Asia between 500 and 1500 m of altitude. In addition to the importance of the genus in traditional medicine, its flowers ranging from pale yellow to deep orange make of it economically valuable ornamental plant. Furthermore, because orchids have complex interactions with specific pollinating insects (Phillips et al. 2011), bacteria (Teixeira da Silva et al. 2015), and with diverse non-mycorrhizal and mycorrhizal endophytic fungi (Selosse 2014; Parthibhan et al. 2017; Novotná et al. 2018; Shah et al. 2019; Meng et al. 2019), they represent an interesting model to study plant-microbe interactions.

Dendrobium species are associated with their fungal community, made of diverse fungal partners (endophytes and epiphytes), that may have beneficial or detrimental effects on

✉ Quentin Favre-Godal
qfavregodal@research.lvmh-pc.com

[1] LVMH recherche, Innovation Matériaux Naturels et Développement Durable, 185 avenue de Verdun, 45800 St Jean de Braye, France

[2] Université de Strasbourg, CNRS, IPHC UMR 7178, Chimie analytique des molécules bioactives et pharmacognosie, F-67000 Strasbourg, France

[3] Agroscope, Swiss Federal Research Station, Plant Protection, 50 Route de Duiller, PO Box1260, Nyon, Switzerland

host plants. The term epiphyte described any organism which grows on the surface of a plant (Yao et al. 2019). The term endophyte, was first introduced by De Bary (Bary 1866), and applies to any organism found within a plant. The word's meaning has since evolved to include all organisms inhabiting plant organs which, at some time in their life, can colonize internal plant tissues without causing apparent harm to their host (Petrini 1991). This definition of fungal endophytes sensu *lato* include consequently the "true" endophytes *stricto* sensu (Mostert et al. 2000), fungi whose colonization never results in visible disease symptoms, and the latent pathogens that may live symptomless in their hosts for some time in their life but may, at a given time, modify their behavior to become pathogens. Some true endophytes have been shown to have beneficial actions on host plants as growth-promoting effects (Shah et al. 2019), to increase host fitness, and to contribute to effective host defense against pathogens (Sarsaiya et al. 2020b; Kamel et al. 2020), herbivores, or abiotic stress (Yamaji et al. 2016). True endophytes may also increase or modulate the content of alkaloids, polysaccharides, saccharides and flavonoids in plants (Zhang et al. 2013; Tian et al. 2014; Taghinasab and Jabaji 2020). Finally, orchids fungal endophytes can be a sustainable source for the development of industrial and pharmaceutical important biomolecules (Sarsaiya et al. 2019b).

Despite the ecological and economical importance of orchids, the relationship with their mycobiome has been insufficiently studied. Furthermore, the molecular mechanisms involved in the establishment and maintenance of the endophytes-host plant relationships are far from being understood (Yan et al. 2019; Favre-Godal et al. 2020; Mattoo and Nonzom 2021). There is a lack of fundamental knowledge on the biochemical interactions between the fungal community and the plant host (Bünger et al. 2020; Trivedi et al. 2020). Such interactions have only been investigated so far for *Dendrobium fimbriatum* association with *Xylaria* species and mycorrhizal fungi (Chen et al. 2013; Xing et al. 2013; Soelistijono et al. 2020).

1.2 Goal of this work

In this study we identified the fungal community of healthy greenhouse *D. fimbriatum* adult plants and characterized the functional potential of each fungal partner on plant growth and/or plant defense.

The fungal community was isolated and identified by a similarity search of their internal transcribed spacers 1 and 2 plus the 5.8S (ITS) nuclear ribosomal DNA sequence with sequences deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

To characterize the action of each isolate on the adult host plant, co-culture experiments with evaluation of the establishment and maintenance of the relationship, and the neutral/positive/negative effects on the host were the first-choice experiments. Usually, it is done by with protocorms/plantlets treated with fungal elicitor solution (supernatant of several days old fungal liquid culture) (Shah et al. 2019; Chand et al. 2020) or pure fungal culture (Sarsaiya et al. 2020b). Unfortunately, co-culture experiments can be challenging with the high numbers of fungal candidates to evaluate and the difficulties to obtain axenic plants as orchids tend to accumulate a high number of fungal endophytes. To overcome these issues, a preliminary ex situ evaluation of the ability of fungal isolates to produce different plant-microbe features such as metabolites or enzymes known to be involved in plant growth and/or plant defense (phytohormones, siderophores, antimicrobial and hydrolytic enzymes) as well as their antifungal activity on pathogenic fungal species of orchids (*Botrytis cinerea*, *Colletotrichum gloeosporioides*, and *Fusarium oxysporum*), was performed (Dolatabad et al. 2017; Bilal et al. 2018; Shah et al. 2019; Chand et al. 2020).

Finally, the characterization of the isolates as beneficial or detrimental partner based on these results and the limitation of such approach to select an appropriate fungal partner for further analysis were discussed.

2 Materials and methods

2.1 Isolation, identification, and molecular systematics of fungal isolates

Three commercial pot-cultured of *Dendrobium fimbriatum* var. *oculatum* Hook. were obtained from the Phrao Orchids Nursery green house in Chiang Mai, Thailand.

The cultivable part of the fungal community associated with these apparently healthy mature plants was isolated by sampling plant material from healthy roots, shoots, leaves and flowers. The fresh tissues, taken randomly from two pots, were first rinsed thoroughly under a gentle stream of tap water for 2 h (non-aggressive removal of surface particles without chemicals). Three samples per organs were then washed in four successive baths of sterile distilled water under a laminar flow hood during 5 min each, then sectioned (longitudinally) using a sterile blade. Squares of 9 mm^2 were placed each on Petri dish containing potato dextrose agar (PDA) amended with chlortetracycline hydrochloride (25 mg/L; Sigma ref. 26,430).

Plates were inspected daily for the emergence of fungi over 3 weeks. Emerging fungi were isolated in pure culture and grown on PDA at room temperature. The isolated fungal strains are stored at 4 °C in the dynamic mycotheca of Agroscope (www.mycoscope.ch) in vials containing a diluted potato dextrose broth (PDB) solution in sterile water (1:4).

A sample of 0.5 cm^3 of each fungal pure culture was placed in an Eppendorf tube containing 500 µl of cetyl trimethyl ammonium bromide buffer (CTAB 1x), and fungal genomic DNA extractions were performed following Inglis et al. (2018). Fungal strains were characterized by amplification and sequencing of their internal transcribed spacers 1 and 2 (ITS) plus the 5.8S of ribosomal DNA (rDNA), using the primers ITS1F and ITS4 (5'-CTTGGTCATTTAGAGGAAGTAA-3′ and 5'-TCCTCCGCTTATTGATATGC-3′ respectively); (http://sites.biology.duke.edu/fungi/mycolab/primers.htm). Polymerase chain reaction (PCR) used the following program: Initial denaturation at 95 °C for 2 min; 20 cycles including 30 s of denaturation at 94 °C, 30 s of primer annealing at 55 °C, 30 s of extension at 72 °C, and a final extension at 72 °C for 10 min. Purification and Sanger's sequencing of the PCR products were performed by Fasteris Sa (Geneva, Switzerland; https://fasteris.com/). Sequencing was performed in both directions using the same primers as for amplification and using Sanger's method. The obtained ITS sequences were deposited in GenBank.

Once trimmed, a first sequence similarity search (BLAST®; https://blast.ncbi.nlm.nih.gov/) in GenBank (National Center for Biotechnology Information; (Benson et al. 2018)) was performed using the "blastn" (Megablast) option excluding "uncultured/environmental sample sequences". A second similarity search was done limiting the search to "sequences from type material". When blast top scores were equally high for several GenBank sequences we favored sequences published in taxonomic studies and/or sequences that were obtained from type material. The method used for the identification of the fungal isolates followed (Hofstetter et al. 2019) with higher cut-offs.

ITS sequences were aligned in MacClade v. 4.08a (Maddison and Maddison 2000). Sequence alignment was performed with the MAFFT v.7.475 program, using default options (Kuraku et al. 2013). Maximum likelihood phylogenetic analyses were conducted in PhyML v. 3.0 (Guindon et al. 2010), with evolutionary model = GTR. Branch robustness was evaluated based on 500 bootstrap replicates, with the same settings as for the search for the most likely tree. Branch support was considered significant when bootstrap values were ≥ 70% (Alfaro et al. 2003).

2.2 Quantification of IAA in liquid medium

The production of IAA and IAA analogues (Indole pyruvic acid and Indole acetamide) by fungal isolates were determined spectrophotometrically with a 96 well plates method adapted from a previous study with Salkowski reagent (Gravel et al. 2007). The reagent is specific for IAA, but also for IAA analogues (Glickmann and Dessaux 1995). Fungal isolates were grown during one week in Petri dishes containing PDA. Liquid cultures were prepared by placing two 9 mm^2 agar plugs of fungal pure cultures in 50 ml falcon tubes with 20 ml of PDB, with or without a 2 mg/ml supplement of *L*-tryptophan. Liquid cultures were done in triplicate. The inoculated broths were incubated in a shaker incubator at 20 °C, 180 rpm for 10 days, and then centrifuged twice at 9000 rpm for 10 min at 20 °C.

Twenty microliters of DMSO, 75 µl of supernatant and 150 µl of Salknowski reagent (49 ml of 70% perchloric acid, 49 ml of deionized water and 2 ml of $FeCl_3$ at 0,5 M) (Gordon and Weber 1951) were mixed in a 96 well plate, and incubated in the dark for 30 min. The optic density was measured at 530 nm using an UV-VIS spectrophotometer (ChromTech-CT 8200). Absorbance of the empty plate was subtracted. The IAA concentration of the extract was quantified against a prepared standard IAA reference scale (1 to 100 µg/ml) constituted of 75 µl of liquid media, 150 µl of Salknowski reagent and 20 µl of IAA solutions in DMSO. Three biological replicates were performed for each experiment.

2.3 Evaluation of siderophore production

Production of siderophores was assessed by a modified chrome azurol S (CAS) method (Milagres and Machuca 1999). After solidification of PDA in Petri dish, the medium was cut into halves, one of which was replaced by CAS-blue agar (Lynne et al. 2011). The plates were inoculated individually on the PDA side with a fungal mycelial plug (9 mm^2) and then incubated at 20 °C for 10 to 15 days, depending on the growth speed. Siderophore production was determined daily by measuring the appearance of the color change on the side containing the CAS-blue indicator. If a red color was observed, the isolate was considered as a producer of siderophores. Three inoculum replicates were made for siderophore production evaluation. Control plates without fungi were also assessed. Positive control was obtained with *Escherichia coli* (ATCC® 25922™).

2.4 Enzymatic profiling

The presence of 19 enzymes for each fungal isolate liquid culture has been confirmed by API-ZYM method (bioMérieux, Craponne, France). The 25 fungal isolates were grown in Petri dishes with PDA medium for one week. Liquid cultures were prepared by placing two 9 mm^2 agar plugs of fungal pre-cultures in 50 ml falcon tubes with 20 ml of PDB. The inoculated broths were incubated in a shaker incubator at 20 °C, under an agitation of 180 rpm for 10 days. After incubation, they were ground with a homogenizer disperser ultra-turrax T25 (Janke & Kunkel®) at 8000 idle revolutions. One milliliter of the shred was resuspended in 4 ml of sterile distilled water and submitted to vortex at 180 rpm for 5 min.

Sixty-five microliters of the fungal suspension in sterile distilled water were added to each cupule of the strips. After

incubation aerobically for 4 h at 37 °C in the dark and addition of manufacturer's reagents (surfactant ZYM A and ZYM B from the API system) in each cupule, the color developed if an enzymatic reaction occurred. Enzymatic activities were classified according to the intensity of color with the API ZYM color reaction chart. All tested strains were examined twice to control the reproducibility of the results.

2.5 Antimicrobial assays

2.5.1 Confrontation assay in petri dish

The confrontation was performed by placing in a PDA Petri dish two 9 mm^2 of agar plugs of one-week isolates fungal pre-cultures and two 9 mm^2 of agar plugs of one-week fungal pre-cultures of phytopathogenic strain. The disposition of the plugs can be seen in Fig. 4a.

The experiment consisted of two Petri dishes replicates, represented by 25 isolates matched with three strains of phytopathogenic fungi (*Botrytis cinerea* MycoBank Agroscope GIK, *Colletotrichum gloeosporioides* MycoBank Agroscope 1015, and *Fusarium oxysporum* MycoBank Agroscope 238). The experiment was evaluated by observing the Petri dishes, using the "Interaction types" as defined by (Bertrand et al. 2013) for analyzing the proportion of antagonism between two paired fungi in culture medium.

2.5.2 Liquid-liquid extraction of fungal liquid culture

Twenty-five liquid cultures were prepared by placing 9 mm^2 of agar plugs of one-week fungal pre-cultures in Erlenmeyer flasks containing 250 ml of PDB and incubating at 20 °C for 21 days. Mycelium was broken with a homogenizer disperser ultra-turrax T25 (Janke & Kunkel®) at maximum speed during 3 mins. Two cycles of liquid extraction were performed using ethyl acetate solvent in a 1:1 ratio of ethyl acetate (EtOAc): broth in the Erlenmeyer flask. The organic phase was collected, filtered, and evaporated using a rotary evaporator at 40 °C resulting in dry EtOAc extracts. The remaining aqueous phase was filtered consecutively with a cotton and a filter paper and finally lyophilized leading to dry aqueous extracts.

2.5.3 Micro-agar dilution method

EtOAc extracts were prepared to obtain three solutions at 40–20-10 mg/ml in DMSO. Aqueous extracts were prepared to obtain final concentrations of 20–10-5 mg/ml in water. Controls were generated in the same way with the commercial fungicide Switch (Cyprodinil 37.5% + Fludioxonil 25%, Syngenta) at 1 mg/ml. Ten microliters of commercial fungicide, 10 µl of EtOAc solutions or 20 µl of aqueous solutions were distributed into sterile 96-well microplates (Greiner, Frickenhausen, Germany). In each well with EtOAc or commercial solutions, 190 µl of PDA maintained at 45 °C was added. In each well with aqueous solution, 180 µl of PDA was added to reach a final volume of 200 µl per well. Each fungal extract was tested in triplicate.

Conidia of *Botrytis cinerea* were collected by vacuum aspiration from 10- day-old sporulating colonies using a filter tip and suspended in a sterile 50 ml Falcon tube containing sterile ultrapure water. The concentration was adjusted to 2×10^6 conidia/ml. Ten microliters of this conidial suspension were added to each well. Same protocol was used for the two other phytopathogenic fungi tested, *Fusarium oxysporum* and *Colletotrichum gloeosporioides.*

The plates were incubated in a growth chamber (60% relative humidity, constant temperature of 21 °C, alternating 16 h day and 8 h night cycles). If no mycelium were observed after 8 days, the extract was considered fungistatic at the concentration tested.

3 Results and discussion

3.1 Molecular identification of isolated fungi

A total of 25 fungal strains were recovered by our isolation procedure from different organs. After molecular identification of the fungal strains isolated by BLAST in GenBank (Table 1), the high majority of the strains belonged to Ascomycota (22) while the three remaining isolates were identified as Mucoromycota (genus *Mucor* [1 isolate] and Basidiomycota (order Agaricales, genus *Coprinopsis* [1] and order Tremellales, genus *Papiliotrema* [1]). Within Ascomycota, 10 strains were Pleosporales (genera *Curvularia* [4 isolates], *Dydimella* [2], *Dydimocyrtis* [1], *Alternaria* [1], *Exserohilum* [1], and *Pseudopithomyces* [1], 5 strains were Hypocreales (genera *Fusarium* [3], and *Trichoderma* [2], and 4 strains Glomerellales (genera *Colletotrichum* [3] and *Plectosphaerella* [1]. The three remaining isolates belonged to Sordariales (genus *Neurospora* [1]) and Eurotiales (genus *Penicillium* [2]). Those isolates are in accordance with the literature as Ascomycota and Basidiomycota are the phyla representing the majority of fungi found aboveground and belowground plant tissues (Trivedi et al. 2020). Compared to stems, leaves, and flowers, more fungi were found in roots. Indeed, the *Curvularia, Coprinopsis, Mortierella,* one *Trichoderma*, one *Penicillium* and one *Fusarium* were isolated from the roots. Isolates with the code OE-RLG were found only in the flowers (Table 1 and Fig. 1). Seven isolated strains (OE 1, OE-2, OE-4, OE-8, OE-27, OE-RLG4 and OE-RLG7) were identified only at the genus level mostly because ITS does not allow to distinguish between very closely related species within species complexes in genera *Alternaria, Curvularia, Fusarium*, *Neurospora* and

Penicillium but also because of poor taxon coverage for *Coprinopsis* (Table 1 and Fig. 1).

Regarding the literature, those genera are common endophytes and have been described previously in *Dendrobium* species (XiQing et al. 2008; Chen et al. 2013; Xing et al. 2013). *Fusarium*, *Phoma* and *Epicoccum* were the dominant isolated endophytes genus in *Dendrobium devonianum* and *D. thyrsiflorum* (Xing et al. 2011). From these two orchid species, *Phoma* and *Rhizopus* were also isolated. *Fusarium* was also the dominant genus isolated from the roots of *D. moniliforme*, followed by *Colletotrichum* sp., *Trichoderma* sp., *Hypoxylon* sp., *Cylindrocarpon* sp. and *Leptosphaerulina* sp. (Shah et al. 2019). *Colletotrichum* strains were also isolated from three *Dendrobium* species: *D. cariniferum*, *D. catenatum* and *D. harveyanum*. Noticeably, it seems that leaves contained the higher number of *Colletrotrichum* species compared to the roots and stems (Ma et al. 2018). Similar observation was made during this study with *Colletotrichum* cf. *cobbittiense* (OE-71) and *C. phyllanthi* (OE-72) isolated from the leaves. In addition to the genera isolated in this work, xylariaceous fungi, corresponding to non-mycorrhizal fungi, were found abundant and diverse in the roots of several *Dendrobium* species (*D. nobile*; *D. faiconeri*; *D. chrysanthum*; *D. aphyllum*; *D. crystallinum; D. fimbriatum*) (Chen et al. 2012, 2013).

No mycorrhizal fungi were isolated due to isolation procedure. In other works, Tulasnellaceae were mainly isolated from identified mycorrhizal orchid roots of *D. fimbriatum* and *D. nobile*. In some plants, few members of the Ceratobasidiaceae and Pluteaceae were also identified (Xing et al. 2013).

Depending on the cultivation procedure (or environmental conditions), the plant life stage, the fungal pool available and the isolation procedures, a different fungal community can be found for a same orchid species (Jia et al. 2016; Jacquemyn et al. 2016; Dastogeer et al. 2020). Therefore, the relatively few isolates described here are obtained from a healthy adult plant cultivated under specific growth conditions and might not be similar for other plant individuals. However, the universality of those genera and their reports among *Dendrobium* genus suggest that they may be common among cultivated *Dendrobium* adult plants.

3.2 Quantification of indole acetic acid (IAA) synthetized by isolated fungi

IAA is the main auxin hormone in plants, having a central role in the regulation of many growth, development, and defense related processes (Davies 2010; Spaepen and Vanderleyden 2011). Phytohormones are key signaling molecules in plant-microbe interaction as they modulate and coordinate cellular and metabolic plant responses associated to the progression of microorganisms across tissues (Boivin et al. 2016; Mehmood et al. 2019). This explain partially why yeast isolated from plants showed higher auxin production compared to the one isolated from the soil (Streletskii et al. 2016).

Production of IAA and IAA analogues from tryptophan independent or dependent pathways were estimated for the fungi isolated from *D. fimbriatum*. Production of IAA without the amino acid addition was at too low concentrations to be detected or considered significant in all isolates (data not shown). However, the indole acetic acid concentration was higher in broths supplemented with tryptophan for all isolates indicating a tryptophan dependent biosynthetic pathway (Fig. 2a).

In this assay, *Colletotrichum cobbittiense* (OE-71) produced very high concentration of IAA and IAA analogues (841 μg/ml ±66), over 11-fold higher than (OE-25) or other isolates. It was necessary to dilute the sample to be in the same absorbance range of the standard curve. In a previous study, fermentation of *C. siamense* in PDB media led to the isolation of plant growth hormone IAA (Munasinghe et al. 2017). Noticeably another species of *Colletotrichum, C. alatae,* isolated from the roots of *D. moniliforme,* was the highest IAA producer in supplemented tryptophan media among the strains tested (Shah et al. 2019). Although OE-71 was originally isolated from the leaves, it does not exclude its presence in the roots, where at least for the Angiosperms, auxin hormones induce the formation and the outgrowth of branching roots.

OE-2, OE-4, OE-5, OE-8, OE-15, OE-20, OE-23, OE-25, OE-26, OE-27, OE-85 and RLG6 produced relatively high amount of IAA and IAA analogues, their average production ranging from 5 to 75 μg/ml. Therefore, most members of the fungal community of *D. fimbriatum* appears to be able to produce IAA and/or IAA analogues. However, some error bars are relatively important due do the variability in the plug agar inoculum per biological replicate.

Production of IAA by similar genus was described in previous published works. For example *Trichoderma atroviride* produced 0.5 and 6.2 μg/ml in medium supplement with tryptophan (Gravel et al. 2007; Colla et al. 2015). *Fusarium* sp. produced between 20 and 45 μg/ml in medium supplement with tryptophan depending on the *Fusarium* isolates (Shah et al. 2019).

Microbial IAA production is considered key in growth promoting activities of beneficial microbes (Khan et al. 2016; Bilal et al. 2018). For example, *Paenibacillus* strains isolated from the meristem of in vitro *Cymbidium eburneum* orchids enhanced the survival and promoted the growth of germinated *Cattleya loddigesii* seedlings, as well as increased the total biomass and number of leaves (Faria et al. 2013). Inhibition of IAA production by application of yucasin (a potent inhibitor of YUCCA, a key enzyme in auxin biosynthesis) reduced the colonization of *Aspergillus awamori* endophyte in maize roots by 52% when applied on the leaves and 66% when applied on the roots. The application of IAA

Table 1 Voucher information for *Dendrobium fimbriatum* var. *oculatum* fungal community. Identifications were based on BLAST top score results (with 100% = positive species identification, with suffix cf. applied for 99–99.9% of similarity with BLAST top score sequence[s] = possibly this species, and aff. For 98–98.9% of similarity = certainly not this species, but related) taking in account sequences similarity and query coverage of our sequences with BLAST top score sequences in GenBank. High similarity cut-offs were used because several of these isolates belong to species complexes (i.e. *Alternaria, Fusarium, Neurospora, Penicillium*) for which even a 100% of ITS similarity does not always allow to distinguish between very closely related species (we reported multiple BLAST top score sequences when corresponding to different species within these species complexes) and because a single mutation might be significant for assigning a different identification to a fungal strain. In the column Phyla, A corresponds to the phylum Ascomycota, B to Basiodiomycota, M to Mucoromycota

Fungal collection ID	Taxon	Source Tissue	GenBank accessions	Most similar sequence in GB	Sequence similarity (%)	Sequence Query coverage (%)	Phyla
OE-01	*Alternaria* sp. Nees 1816	Leave	MW260290	MN820653, MK798494, KU593527 or KU671304	100	100	A
OE-71	*Colletotrichum* cf. *cobbittiense* S. Luo, G. Dong & P. Wong 2018	Leave	MW260276	NR_163538	99.62	100	A
OE-03	*Colletotrichum cymbidiicola* Damm, P.F. Cannon, Crous, P.R. Johnst. & B.S. Weir 2012	Stem	MW260277	NR_165949	100	98	A
OE-72	*Colletotrichum phyllanthi* (H.S. Pai) Damm, P.F. Cannon & Crous 2012	Leave	MW260275	NR_111698	100	100	A
OE-27	*Coprinopsis* sp. P. Karst. 1881	Green root	MW260270	HQ248225	99.78	100	B
OE-06	*Curvularia* cf. *eragrostidis* (Henn.) J.A. Mey. 1959	Root	MW260287	MH859837	99.64	100	A
OE-23	*Curvularia* cf. *nodosa* Y. Marín, Cheew. & Crous 2017	Root	MW260286	NR_154865	99.81	100	A
OE-26	*Curvularia* cf. *pseudobrachyspora* Y. Marín, Cheew. & Crous 2017	Root	MW260285	NR_164423	99.81	99	A
OE-08	*Curvularia* sp. Boedijn 1933	Old root	MW260288	MH414906, HG779011 or HG779002 Type sequences	100	100	A
OE-65	*Didymella anserina* (Marchal) Qian Chen & L. Cai 2015	Leave	MW260292	MH858633	96.6	99	A
OE-17	*Didymella pedeiae* (Aveskamp, Gruyter & Verkley) Qian Chen & L. Cai 2015	Stem	MW260291	MH863383 Type	100	100	A
OE-RLG4	*Didymocyrtis* sp.Vain. 1921	Flower	MW260294	NR_165522	97.27	100	A
OE-69	*Exserohilum* cf. *mcginnisii* A.A. Padhye & Ajello 1986	Inflorescence	MW260289	NR_157456	99.47	99	A
OE-85	*Fusarium* cf. *foetens* Schroers, O'Donnell, Baayen & Hooftman 2004	Root	MW260272	NR_159865	99.41	99	A
OE-04	*Fusarium* sp. 1 Link 1809	Old root	MW260271	MT563420, MT558602, MT358797, MK534502 or MT358781	100	100	A
OE-02	*Fusarium* sp. 2 Link 1809	Leave	MW260274	MH862668 Type or NR_111889	100	100	A
OE-25	*Mortierella* aff. *Sarnyensis* Milko 1973	Old root	MW260268	NR_111565	96.99	100	M
OE-RLG7	*Neurospora* sp.1 Shear & B.O. Dodge 1927	Flower	MW260282	MT367687, MN511320 or MN511320	100	100	A
OE-RLG10	*Papiliotrema* cf. *rajasthanensis* (Saluja & G.S. Prasad) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout 2015	Flower	MW260269	NR_155678	99.57	100	B
OE-28	*Penicillium* cf. *citreosulfuratum* Biourge 1923	Young green root	MW260284	NR_153252	99.07	100	A
OE-RLG8	*Penicillium* cf. *olsonii* Bainier & Sartory 1912	Flower	MW260283	NR_163546	99.62	100	A
OE-RLG6	*Plectosphaerella oligotrophica* T.T. Liu, D.M. Hu & L. Cai 2013	Flower	MW260280	NR_155632	100	100	A
OE-20	*Pseudopithomyces* aff. *Chartarum* (Berk. & M.A. Curtis) Jun F. Li, Ariyaw. & K.D. Hyde 2015	Stem	MW260293	MH860611	98.38	100	A
OE-15	*Trichoderma* cf. *simmonsii* P. Chaverri, F.B. Rocha, Samuels, Degenkolb & Jaklitsch 2015	Leave	MW260279	NR_137297	99.66	100	A
OE-05	*Trichoderma yunnanense* Z.F. Yu & K.Q. Zhang 2007	Old root	MW260278	NR_134419	100	99	A

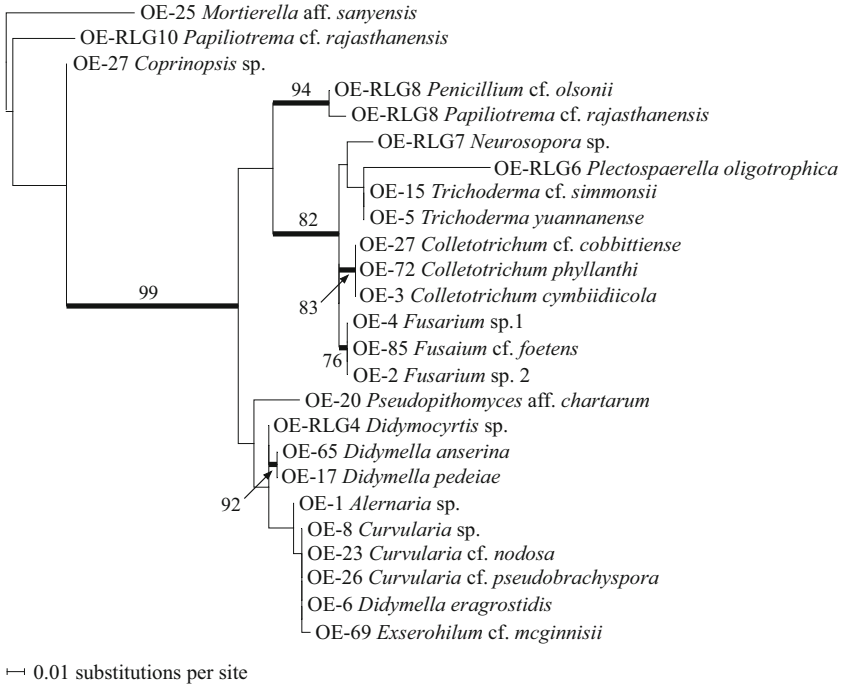


Fig. 1 Phylogeny of the isolated fungal partners of *D. fimbriatum*. The most likely phylogram (−ln = 768.35553) was inferred from analyses of 25 ITS sequences. Sequence analyses comprise 220 characters (including the 5.8S and a small part of the nuclear ribosomal large subunit) after exclusion of ambiguously aligned regions (exclusion of the full ITS 1 and almost all ITS 2 except eight characters). The sequence of the fungal strain identified by BLAST as *Mortierella* aff. *Sarnyensis* was used as outgroup sequence (James et al., 2006). Branches that received significant support are in bold black with bootstrap values indicated along the branches. Isolates with the code OE-RLG were found only in the flowers

restored the ability of *Aspergillus awamori* to colonize maize roots and significantly improved various growth parameters (Mehmood et al. 2019).

On the other hand, microbial IAA may act as a virulence factor during disease development (Fu et al. 2015). Indeed, microbial IAA can stimulate plant cell growth during infection, suppress host defenses and regulate virulence gene expression (Kunkel and Harper 2018; Djami-Tchatchou et al. 2020). IAA can be synthesized from tryptophan via the intermediate indole-3-acetamide (IAM) or via the intermediate

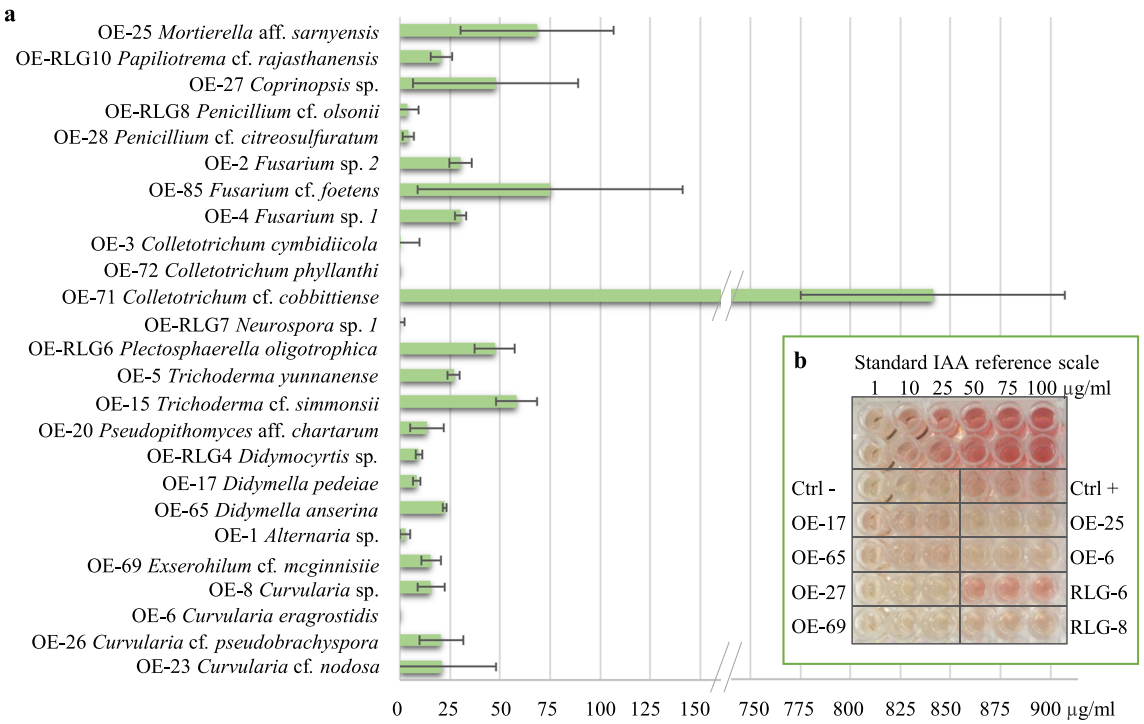


Fig. 2 **a**. Production of IAA and IAA-related compounds by isolated fungi in liquid cultures with PDB supplemented with 2 mg/ml of *L*-tryptophan as an IAA precursor. Each value represents a mean of three replicates ± SE. expressed in µg/ml of IAA equivalent. **b**. Example of results obtained in microplate after incubation with the reagent

indole pyruvic acid (IPA). IAM pathway was found in the cereal rust pathogen *Puccinia graminis f.* sp. *tritici.* and demonstrated by means of transient silencing that one gene of the pathway is required for full pathogenicity (Yin et al. 2014). Transgenic fungal pathogens of *Orobranche aegyptiaca*, *Fusarium oxysporum* and *F. arthrosporioides* overproducing IAA have enhanced virulence, which results in a decrease in the number and size of plant shoots (Cohen et al. 2002). Strikingly, the highest producer of IAA in this assay, OE-71, is from the genera *Colletotrichum*, from which several species were described as important phytopathogens of orchids (Kadir et al. 2020; Park et al. 2020). OE-71 was obtained from the leaves of *D. fimbriatum*, parts of the plant where the anthracnose disease is predominantly observed.

All those data support a primordial role of microbial IAA in plant-microbe interaction (beneficial or detrimental), particularly at the colonization step leading to plant growth stimulation or modification of basal plant defense mechanisms (Goyal and Kalia 2020).

3.3 Production of siderophores by isolated fungi

Siderophores are low molecular-weight compounds (less than 1 kDa), that have high affinity for ferric iron. Those iron-chelating molecules are produced by numerous organisms to sequester preferably iron or other metals from the environment allowing their future use for correct development. Siderophores are important in various ecological phenomena including iron biogeochemical cycling in soils, pathogen competition, plant growth promotion and regulation, communication, virulence, and oxidative stress processes during plant-microbe interaction (Albelda-Berenguer et al. 2019; Trivedi et al. 2020).

As siderophore production strongly coincides with a plant-associated lifestyle (Stringlis et al. 2018), extracellular siderophore secretion by isolated fungi have been measured by the CAS-blue agar assay (Fig. 3 and Online Resource 1).

In the present study, fifteen out of the 25 fungal species isolated from *Dendrobium fimbriatum* plants produced siderophores (OE-1, 3, 4, 5, 8, 17, 20, 23, 26, 28, 69, 85, RLG-7 and RLG-8) (Online Resource. 1 and Fig. 3). *Fusarium* cf. *foetens* (OE-85), *Fusarium* sp.2 (OE-18) and *Penicillium* cf. *citreosulfuratum* (OE-28) were clearly identified as siderophore producers due to a strong CAS media coloration. Species of the genus *Fusarium* and *Trichoderma* were previously designated as siderophore producers (Dolatabad et al. 2017; Bilal et al. 2018), whereas for *Penicillium* spp., siderophore production has been shown to be strain dependent (Shi et al. 2017). Not surprisingly, the strongest producers were isolated from the roots of *D. fimbriatum*, however, over half of the isolates in this work are producing extracellular siderophores, suggesting siderophore production a common ability for plant-associated microbes. No phylogenetic placement links could be made in this study. *Curvularia eragrostidus* or *Fusarium* sp. *2* did not produce siderophores whereas other species from the same genus did.

Microbial siderophore production can confer plant resistance to biotic stress. They can alter the microbial community by resource-competition (Kramer et al. 2020) and induction of the plant induced systemic resistance that would control the growth of soil-borne pathogens neighbors (Kundan et al. 2015; Igiehon and Babalola 2018). In parallel, siderophores reduce the plant oxidative stress, facilitate plant iron acquisition (Bar-Ness et al. 1992; Dellagi et al. 2009) and so confer resistance to abiotic stress (Choudhary et al. 2016; Kowalczyk and Latowski 2018). Consequently, they would promote plant growth as iron deficiency in plants causes chlorosis and growth defects (Hindt and Guerinot 2012; López-Millán et al. 2013). Such siderophore producing endophytic fungal strains are consequently considered as beneficial for plant health (Yamaji et al. 2016).

However, siderophores also play a pathogenesis effector role (Ruddat et al. 1991; Haas et al. 2008; Taguchi et al. 2010; Aznar and Dellagi 2015; Kramer et al. 2020). For example, mutants of *Colletotrichum graminicola* (maize pathogen), unable to produce siderophores were deficient in vegetative growth under iron-limiting conditions, conidiation, reactive oxygen species tolerance, cell wall integrity, and are though not virulent (Albarouki et al. 2014). Siderophore production is considered an essential virulence factor for some pathogenic species (Ruddat et al. 1991; Haas et al. 2008; Taguchi et al. 2010; Aznar and Dellagi 2015; Kramer et al. 2020).

Despite the clear involvement of siderophores in plant-microbe interaction and the high number of producers among the fungal community studied here, the incomplete understanding of siderophore production and utilization in such interaction prevent the direct description of siderophore-producers as beneficial or detrimental.

3.4 Anti-phytopathogenic activity of isolated fungi

Several fungal phytopathogens are reducing the quality and survival of Orchidaceae plants. *Botrytis cinerea* is responsible of botrytis petal blight, which is one of the most destructive and economically important diseases affecting greenhouse grown ornamental plants (Bika et al. 2020). *Colletotrichum gloeosporioides* causes anthracnose and has been significant in *Dendrobium* production losses (McMillian Jr 2011). Finally, species of the *Fusarium* complexes such as *oxysporum, proliferatum, solani, subglutinans and fractiflexum*, are reported to cause foliar and root diseases (*Fusarium* wilt) on *Dendrobium*, *Cymbidium*, *Phalaenopsis* and *Cattleya* plant species (Srivastava et al. 2018). The number of *Fusarium* diseases in orchids is increasing, leading to

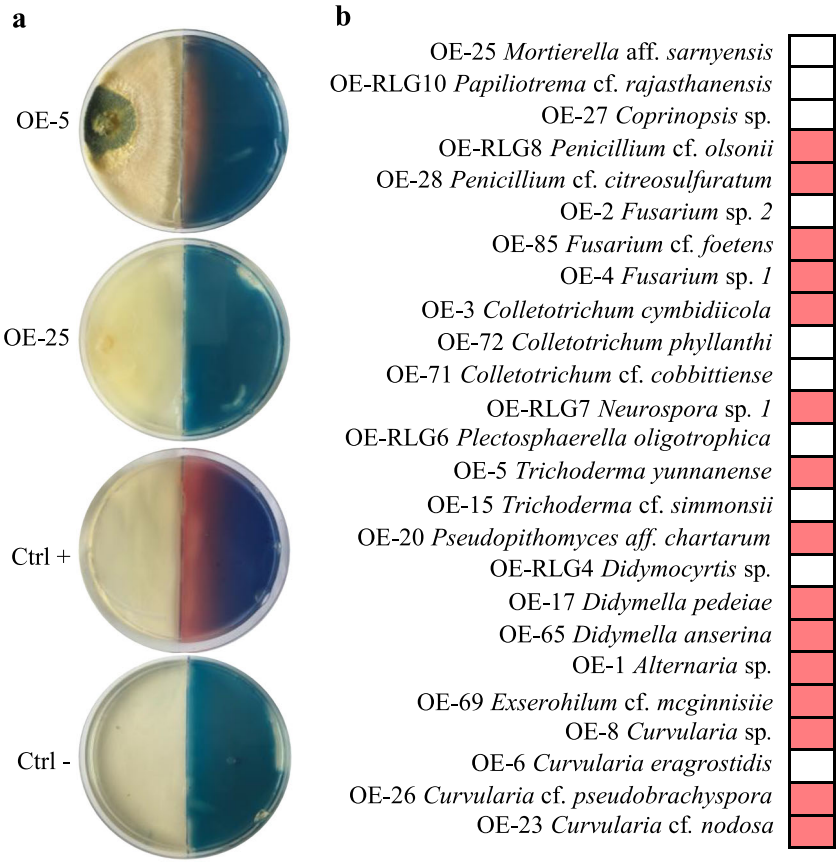


Fig. 3 **a**. Examples of results obtained with agar Petri dish containing CAS-blue agar on the right side when PDA media on the left side was inoculated with: *Trichoderma asperellum* (OE-5), picture was taken after 10 days of culture; *Mortierella* sp. (OE-25), picture was taken after 12 days of culture; *Escherichia coli*, positive control, picture was taken after 3 days of culture; no inoculum, negative control, picture was taken after 20 days of culture. Orange-Red colors indicate siderophores. **b**. Siderophores production by the isolated strains. Orange color indicate siderophores presence detected by the CAS-blue agar assay. Experience was done in triplicate

consider pathogenic *Fusarium* spp. as a major limiting factor for the production of high quality orchids (Srivastava et al. 2018; Jain et al. 2021).

To evaluate the anti-phytopathogenic activity of the fungi isolated in this work, confrontation assays in Petri dish with each isolate versus a main orchid phytopathogen (*Botrytis cinerea*, *Colletotrichum gloeosporiodes* and *Fusarium oxysporum*) were performed (Fig. 4a). In addition, their ethyl acetate (EtOAc) and aqueous extracts obtained by liquid-liquid extraction of a liquid culture were tested by micro-agar dilution method against those same three main orchid phytopathogens (Fig. 4b).

The EtOAc extract of both *Trichoderma* sp. (OE-5 and OE-15, Table 1) and of two *Fusarium* isolates (OE-2 and OE-18) had anti-phytopathogenic fungal activities against all the three species tested (Fig. 4c). The EtOAc extract of *Fusarium* sp. *1* (OE-4) had activity against *Botrytis cinerea* and *Colletotrichum gloeosporioides* but no activity against *Fusarium oxysporum.*

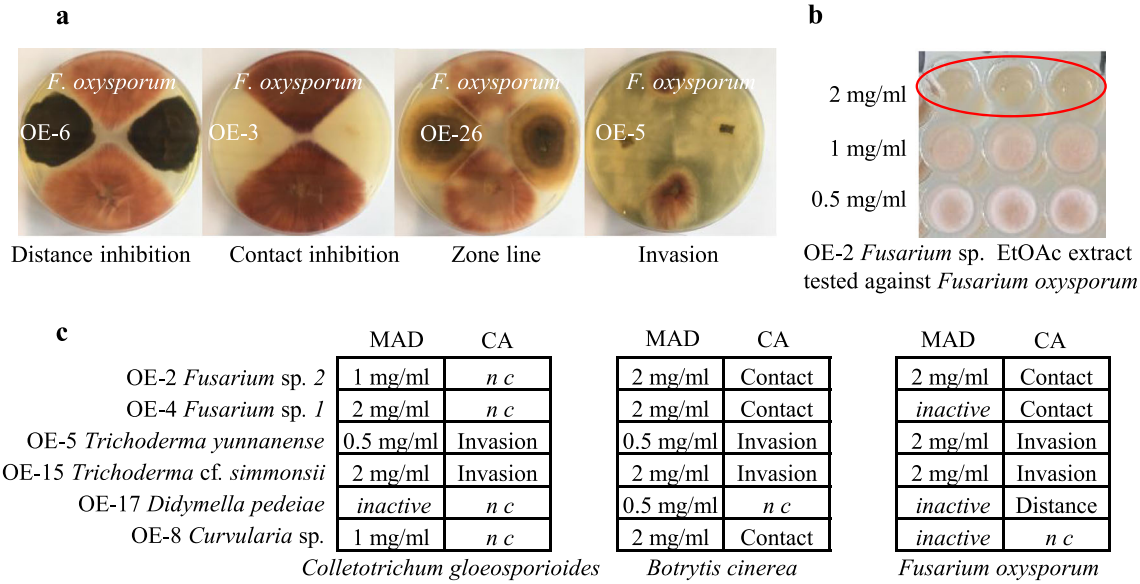
Those results are consistent with the literature as *Trichoderma* species, and to a smaller extent, non-pathogenic *Fusarium*, are known for their anti-phytopathogenic fungal activities due to their secondary metabolites, plant growth promotion, and local, as well as systemic, induced resistance (Al-Ani 2019; Saravanakumar and Wang 2020; Khan et al. 2020). For example, dendrobine, an alkaloid initially found in *D. nobile,* was isolated from *T. longibrachiatum* and had antibacterial activity against *Bacillus subtilis*, *Bacillus mycoides*, and *Staphylococcus* species (Sarsaiya et al. 2020a). Harzianic acid, a tetramic acid compound from *T. harzianum,* showed antifungal activity against *Pythium irregulare*, *Sclerotinia sclerotiorum*, and *Rhizoctonia solani* (Vinale et al. 2009) as well as plant growth promotion activities, probably due to its Fe(III)-binding activity (Vinale et al. 2013).

All the *Curvularia* EtOAc extracts had no anti-phytopathogenic fungal activities except for one *Curvularia* isolate (OE-8) extract, active against *B. cinerea* and *C. gloeosporioides.*

Didymella pedeiae (OE-17) EtOAc extract had strong anti-phytopathogenic fungal activity (0.5 mg/ml) against *B. cinerea* (Fig. 4c).

The aqueous extracts were inactive at the concentration tested. This is probably due to the low quantity of fungal secondary metabolites recovered compared to the culture media ingredients remaining in the aqueous phase.

Regarding the confrontation assays, all *Trichoderma* strains grown completely over the phytopathogenic fungi covering the surface of the medium whereas the *Fusarium* strains showed a contact inhibition profile with no dominance over



	Colletotrichum gloeosporioides		Botrytis cinerea		Fusarium oxysporum	
	MAD	CA	MAD	CA	MAD	CA
OE-2 *Fusarium* sp. *2*	1 mg/ml	*n c*	2 mg/ml	Contact	2 mg/ml	Contact
OE-4 *Fusarium* sp. *1*	2 mg/ml	*n c*	2 mg/ml	Contact	*inactive*	Contact
OE-5 *Trichoderma yunnanense*	0.5 mg/ml	Invasion	0.5 mg/ml	Invasion	2 mg/ml	Invasion
OE-15 *Trichoderma* cf. *simmonsii*	2 mg/ml	Invasion	2 mg/ml	Invasion	2 mg/ml	Invasion
OE-17 *Didymella pedeiae*	*inactive*	*n c*	0.5 mg/ml	*n c*	*inactive*	Distance
OE-8 *Curvularia* sp.	1 mg/ml	*n c*	2 mg/ml	Contact	*inactive*	*n c*

Fig. 4 **a**. Pictures of confrontation assays against *Fusarium oxysporum*. **b**. Picture of micro-agar dilution method in 96 well plates of OE-2 EtOAc extract against *Fusarium oxysporum*. Red circle shows the inhibition at 2 mg/ml for the three replicates. **c**. Active EtOAc extracts concentrations detected by micro-agar dilution method (MAD) and the results of confrontation assays (CA) of the corresponding strain. *n c:* not conclusive results

the pathogenic species (Fig. 4c). *Trichoderma* after a week invaded and sporulated over the phytopathogen revealing its mycoparasitism (Sood et al. 2020).

For a better observation of the physical interactions of the hyphae, the replication of this confrontation assay at a smaller scale on a microscope slide covered in a thin layer of nutrient agar medium would be interesting (Hajieghrari and Giglou 2008; Djami-Tchatchou et al. 2020).

The EtOAc extract of OE-17 was inactive against *Fusarium oxysporum* but when OE-17 is in co-culture with this phytopathogen it was able to partially inhibit its growth. It might be due to induced antifungal mechanisms. In addition to constitutive antifungal productions, co-culture can induced the production of antimicrobials (Deshmukh et al. 2018; Arora et al. 2020; Christiansen et al. 2020). As micro-agar dilution assay does not measure such mechanism, the use of each methodology is complementary for our primary screening.

Only few fungi were able to inhibit the growth of the pathogenic strains studied here. This experiment is therefore interesting to evaluate and differentiate the functional potential of each isolate. Noticeably, isolates with anti-phytopathogenic activity were obtained from the leaves and roots, which are parts often exposed to fungal diseases, suggesting their beneficial impact for the host plant.

3.5 Secretion of hydrolytic enzymes by isolated fungi

Most fungal partner have the genetic capacity to produce enzymes (cellulases, xylanases and pectinases) that degrade the major structural polysaccharides found in the cell wall (cellulose, xylan and pectin) in order to enter the host cell (King et al. 2011).

The capabilities of fungal endophytes to produce specific enzymes can consequently be used for assessing their metabolic diversity, giving indication on their ecological role.

API-ZYM galleries were designed for semi-quantitative test of 19 constitutively expressed lipid, protein and carbohydrate-degrading enzymes of non-pretreated bacteria (e.g. Humble, King and Phillips 1977). Relatively few studies investigated the enzymograms of filamentous fungi using this technique (Bidochka et al. 1999; Tekere et al. 2001; Knapp and Kovács 2016; Żukiewicz-Sobczak et al. 2016). Cleverly, Knapp and Kovács (2016) used API-ZYM system to study interspecific metabolic diversity and functional heterogeneity of roots non-sporulating endophytic fungi. An adapted version of their protocol was used to highlight similarities or differences in enzymes production and therefore in their ecological functions (Fig. 5).

The production of hydrolytic enzymes was slightly variable among the fungal isolates but appeared to be unlinked to their phylogenetic placement or their origin tissue. In general, similar results with (Knapp and Kovács 2016) were obtained. Almost no trypsin (except for OE-23), lipase (except for OE-27), α-fucosidase, α-mannosidase, chymotrypsin and cystine arylamidase activities were detected from hyphal fragments and extracellular medium of the tested fungi.

In opposition, activities of esterase (C4), esterase lipase (C8), acid and alkaline phosphatase and β-glucosidase were

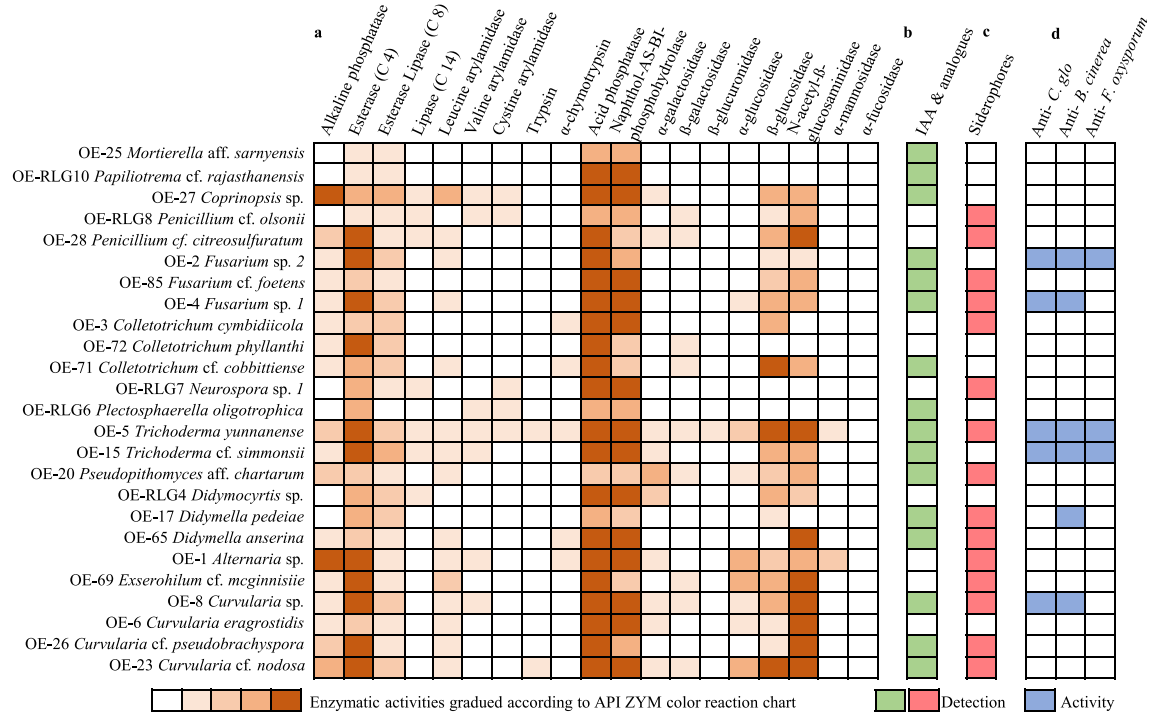


Fig. 5 Occurrence of plant-microbe features in all the isolates. a. Enzymatic activities graded according to the intensity of color with the API ZYM color reaction chart. ITS phylogeny tree of Fig. 1 is used as entry for this table to facilitate the reading of the results. All tested strains were examined twice to control the reproducibility of the results. b. IAA related compounds detection by colorimetric assay. c. Detection of siderophores by the CAS-blue agar assay. d. Anti-phytopathogenic activity evaluated by micro-agar dilution method. Representation inspired by Knapp and Kovács (2016)

strong with a clear detection for almost all isolates (Fig. 5a). All enzymograms were quite similar except for *Mortierella* aff. *Sarnyensis.* (OE-25) and *Didymella pedeiae* (OE-17) that produced fewer different enzymes compare to other members of the fungal community.

Species with esterase, cellulase, amylase, protease acid and alkaline phosphatase enzyme activities might play a role in the promotion of plant growth by decomposing organic matter, increasing phosphate availability and nutrient mineralization (Gianfreda 2015). They can also act as biocontrol agents for pathogens or stimulators of the plant immune system due to the release of partially enzymatic degraded wall constituents (Lionetti and Métraux 2014). The degradation products of the major structural polysaccharides found in the cell wall might also be used as nutrients because endophytes can become saprobes after the cell plant death or pathogens, depending on the plant-host conditions (King et al. 2011).

In addition to the nutrition role of phosphatase enzymes, it has been suggested that they also play important roles in proliferation, differentiation, adhesion, virulence and plant-infection (Freitas-Mesquita and Meyer-Fernandes 2014). For example, Cdc14 protein phosphatases play an important role in plant infection by several fungal pathogens such as *Aspergillus flavus* (Yang et al. 2018).

The ability to produce hydrolytic enzymes is common between beneficial, saprobes and detrimental fungi but the substrates specificities and kinetics might differ. Furthermore, fungal enzymes production is a substrate induced and dependent process. The enzymograms obtained in this work are consequently partial, and *D. fimbriatum* fungal community might produce several other enzymes of interest not evaluated in this preliminary study (like cutinase and lignin-modifying enzymes for example). API ZYM test remains a starting procedure to quickly study some enzymes production capability of fungi, but further analyses are needed as it could not help us to differentiate and characterize the ecological functions of the isolates studied here.

3.6 Characterization of isolated fungi as beneficial or pathogenic strains

It is important to notice that antimicrobials, phytohormones, siderophores and hydrolytic enzymes production are mechanisms used by both beneficial and pathogenic strains (cf. results discussion). Furthermore, the establishment of both plant-beneficial and plant-pathogenic microbe interactions, and the molecular communication strategies involve a balance of multiple factors which are often overlapping (Stringlis et al. 2018; Favre-Godal et al. 2020; Trivedi et al. 2020). In addition to the complexity of such interactions, general fungi of the mycobiome can easily shift from mutualist to parasite depending on the plant's health and environmental conditions,

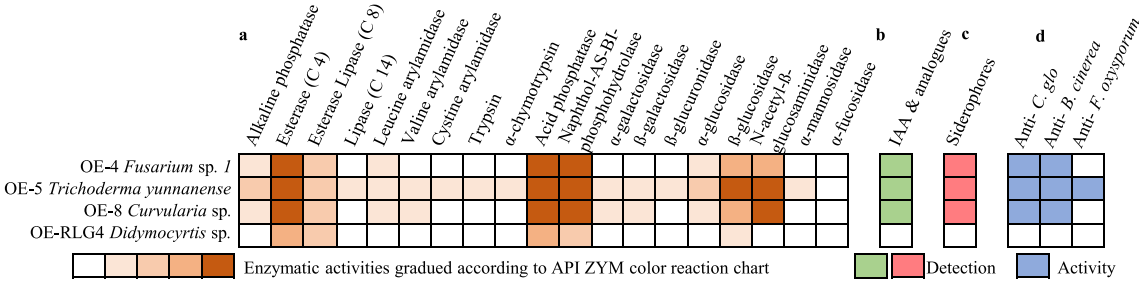


Fig. 6 Occurrence of plant-microbe features in four isolates. Three isolates predicted to have strong interactions with the host plant (OE-4; OE-5 and OE-8) and one isolate (OE-RLG4) predicted to have very few interactions. a. Enzymatic activities were graded according to the intensity of color with the API ZYM color reaction chart. b. IAA related compounds detection by colorimetric assay. c. Detection of siderophores by the CAS-blue agar assay. d. Anti-phytopathogenic activity evaluated by micro-agar dilution method

requiring precaution in the description of beneficial partner (Tejesvi and Pirttilä 2018; Sarsaiya et al. 2019b; Jain et al. 2019). This is probably why, during experimental inoculations, the influence of a fungal root endophyte on the host plant could be controversial, with plant responses ranging from negative to positive (Mayerhofer et al. 2013).

It is then not surprising to see some *Trichoderma* species, frequently used as biological control agents and biofertilizers to control plant diseases and enhance crop yields (Alfiky and Weisskopf 2021) while others are described as pathogens (Li Destri Nicosia et al. 2014), with *T. longibrachiatum* causing black circular spots on *Dendrobium nobile* leaves (Sarsaiya et al. 2019a). Another example could be *Fusarium* spp., some are described as endophytes with beneficial effects (Hiruma et al. 2016; Hiruma 2019), like *F. oxysporum* reducing disease caused by vascular pathogens such as *Verticillium dahliae* and conferring protection against root pathogens like *Pythium ultimum* (de Lamo and Takken 2020), whereas the same species, *F. oxysporum* is an important fungal pathogen of orchids.

For all of those reasons, the plant-microbe features studied here should not be considered alone, out of a specific interaction, to categorize a strain of the mycobiome as beneficial or detrimental but rather used to characterize and select fungi with important plant-lifestyle behavior for further considerations.

In this context epiphytes, "true endophytes" regarding the definition of (Mostert et al. 2000) and probably latent pathogens have been isolated during this work. To differentiate epiphytes and endophytes, a stronger asepsis protocol can be performed. To differentiate "true endophytes" and latent pathogens, the plant-microbe features studied here are only preliminary results, needed to be complemented. To date, to unambiguously categorize isolated fungal orchids partners from latent pathogens to mutualistic symbionts, phenotypic evaluations with co-cultures experiments are still necessary (Sarsaiya et al. 2020b).

With the increasing knowledge on effector genes, comparing the genomes of the isolates and their putative effector genes (coding for putative host-specific virulence proteins), could become a strategy to differentiate beneficial to latent pathogens of a large set of fungal isolates. Indeed, endophytes, even if they have a common set of conserved putative effector genes with pathogenic strains, they appear to carry a fewer number and almost no host-plant specific effectors (Dam et al. 2016).

In the present study, three isolates out of twenty-five produced significantly all the evaluated plant-microbe features. *Fusarium* sp. (OE-4), *Trichoderma yunnanense* (OE-5), and *Curvularia* sp. (OE-8) are the fungal isolates from *D. fimbriatum* producing all siderophores, phytohormones and anti-phytopathogenic compounds (Fig. 6).

Noticeably, they were isolated from the roots, where plant-microbe interactions have been extensively documented. Those results designate them as fungal partners having potentially, strong interactions with the host plant, making of them candidates for further integrative approaches such as experimental modelling through co-cultivation experiments.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13199-021-00786-0.

Acknowledgments We would like to thank Léa Mazina for her logistic support, Marine Bellanger for her proofreading, and Eric Remolif and Sylvia Dusonchet for their expertise and work on orchid growth.

Funding This research was financially supported by Guerlain to contribute in the understanding of microbe-plant relationship and preservation of orchids.

Data availability ITS sequences can be found in Genbank with the accession's numbers of Table 1. The fungal strains isolated are stored at 4 °C in the dynamic mycotheca of Agroscope (www.mycoscope.ch).

Declarations

Conflicts of interest/competing interests Authors declare they have no financial interests and no conflicts of interest to declare that are relevant to the content of this article.

References

Al-Ani LKT (2019) Secondary metabolites of non-pathogenic *Fusarium*: scope in agriculture. In: Singh HB, Keswani C, Reddy MS et al (eds) Secondary metabolites of plant growth promoting

Rhizomicroorganisms: discovery and applications. Springer, Singapore, pp 59–76

Albarouki E, Schafferer L, Ye F, Wirén N, Haas H, Deising HB (2014) Biotrophy-specific downregulation of siderophore biosynthesis in *Colletotrichum graminicola* is required for modulation of immune responses of maize. Mol Microbiol 92:338–355. https://doi.org/10.1111/mmi.12561

Albelda-Berenguer M, Monachon M, Joseph E (2019) Chapter five - Siderophores: from natural roles to potential applications. In: Gadd GM, Sariaslani S (eds) Advances in Applied Microbiology. Academic Press, pp. 193–225

Alfaro ME, Zoller S, Lutzoni F (2003) Bayes or bootstrap? A simulation study comparing the performance of Bayesian Markov chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. Mol Biol Evol 20:255–266. https://doi.org/10.1093/molbev/msg028

Alfiky A, Weisskopf L (2021) Deciphering *Trichoderma*-plant-pathogen interactions for better development of biocontrol applications. J Fungi Basel Switz 7:61. https://doi.org/10.3390/jof7010061

Arora D, Gupta P, Jaglan S, Roullier C, Grovel O, Bertrand S (2020) Expanding the chemical diversity through microorganisms co-culture: current status and outlook. Biotechnol Adv 40:107521. https://doi.org/10.1016/j.biotechadv.2020.107521

Aznar A, Dellagi A (2015) New insights into the role of siderophores as triggers of plant immunity: what can we learn from animals? J Exp Bot 66:3001–3010. https://doi.org/10.1093/jxb/erv155

Bar-Ness E, Hadar Y, Chen Y, Shanzer A, Libman J (1992) Iron uptake by plants from microbial siderophores: a study with 7-nitrobenz-2 oxa-1,3-diazole-desferrioxamine as fluorescent ferrioxamine B analog. Plant Physiol 99:1329–1335. https://doi.org/10.1104/pp.99.4.1329

de Bary A (1866) Morphologie und physiologie der pilze, flechten und myxomyceten. W. Engelmann, Leipzig. https://doi.org/10.5962/bhl.title.120970

Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Ostell J, Pruitt KD, Sayers EW (2018) GenBank. Nucleic Acids Res 46:D41–D47. https://doi.org/10.1093/nar/gkx1094

Bertrand S, Schumpp O, Bohni N, Bujard A, Azzollini A, Monod M, Gindro K, Wolfender JL (2013) Detection of metabolite induction in fungal co-cultures on solid media by high-throughput differential ultra-high pressure liquid chromatography-time-of-flight mass spectrometry fingerprinting. J Chromatogr A 1292:219–228. https://doi.org/10.1016/j.chroma.2013.01.098

Bidochka MJ, Burke S, Ng L (1999) Extracellular hydrolytic enzymes in the fungal genus *Verticillium*: adaptations for pathogenesis. Can J Microbiol 45:856–864. https://doi.org/10.1139/w99-085

Bika R, Baysal-Gurel F, Jennings C (2020) *Botrytis cinerea* management in ornamental production: a continuous battle. Can J Plant Pathol 0:1–21. https://doi.org/10.1080/07060661.2020.1807409

Bilal L, Asaf S, Hamayun M, Gul H, Iqbal A, Ullah I, Lee IJ, Hussain A (2018) Plant growth promoting endophytic fungi *Aspergillus fumigatus* TS1 and *Fusarium proliferatum* BRL1 produce gibberellins and regulates plant endogenous hormones. Symbiosis 76:117–127. https://doi.org/10.1007/s13199-018-0545-4

Boivin S, Fonouni-Farde C, Frugier F (2016) How auxin and cytokinin phytohormones modulate root microbe interactions. Front Plant Sci 7:1240. https://doi.org/10.3389/fpls.2016.01240

Bünger W, Jiang X, Müller J, Hurek T, Reinhold-Hurek B (2020) Novel cultivated endophytic *Verrucomicrobia* reveal deep-rooting traits of bacteria to associate with plants. Sci Rep 10:8692. https://doi.org/10.1038/s41598-020-65277-6

Cakova V, Bonte F, Lobstein A (2017) *Dendrobium*: sources of active ingredients to treat age-related pathologies. Aging Dis 8:827–849. https://doi.org/10.14336/AD.2017.0214

Chand K, Shah S, Sharma J, Paudel MR, Pant B (2020) Isolation, characterization, and plant growth-promoting activities of endophytic fungi from a wild orchid *Vanda cristata*. Plant Signal Behav 15:1744294. https://doi.org/10.1080/15592324.2020.1744294

Chen J, Wang H, Guo S-X (2012) Isolation and identification of endophytic and mycorrhizal fungi from seeds and roots of *Dendrobium* (Orchidaceae). Mycorrhiza 22:297–307. https://doi.org/10.1007/s00572-011-0404-0

Chen J, Zhang L-C, Xing Y-M, Wang YQ, Xing XK, Zhang DW, Liang HQ, Guo SX (2013) Diversity and taxonomy of endophytic xylariaceous fungi from medicinal plants of *Dendrobium* (Orchidaceae). PLoS One 8:e58268. https://doi.org/10.1371/journal.pone.0058268

Choudhary DK, Kasotia A, Jain S, Vaishnav A, Kumari S, Sharma KP, Varma A (2016) Bacterial-mediated tolerance and resistance to plants under abiotic and biotic stresses. J Plant Growth Regul 35:276–300. https://doi.org/10.1007/s00344-015-9521-x

Christiansen L, Alanin KS, Phippen CBW, Olsson S, Stougaard P, Hennessy RC (2020) Fungal-associated molecules induce key genes involved in the biosynthesis of the antifungal secondary metabolites nunamycin and nunapeptin in the biocontrol strain *Pseudomonas fluorescens* In5. Appl Environ Microbiol 86:e01284–e01220. https://doi.org/10.1128/AEM.01284-20

Cohen BA, Amsellem Z, Maor R, Sharon A, Gressel J (2002) Transgenically enhanced expression of indole-3-acetic acid confers hypervirulence to plant pathogens. Phytopathology 92:590–596. https://doi.org/10.1094/PHYTO.2002.92.6.590

Colla G, Rouphael Y, Mattia ED et al (2015) Co-inoculation of *Glomus intraradices* and *Trichoderma atroviride* acts as a biostimulant to promote growth, yield and nutrient uptake of vegetable crops. J Sci Food Agric 95:1706–1715. https://doi.org/10.1002/jsfa.6875

Dastogeer KMG, Tumpa FH, Sultana A, Akter MA, Chakraborty A (2020) Plant microbiome–an account of the factors that shape community composition and diversity. Curr Plant Biol 23:100161. https://doi.org/10.1016/j.cpb.2020.100161

Davies PJ (ed) (2010) Plant hormones: biosynthesis, signal transduction, action!, 3rd edn. Springer, Netherlands. https://doi.org/10.1007/978-1-4020-2686-7

De Lamo FJ, Takken FLW (2020) Biocontrol by *Fusarium oxysporum* using endophyte-mediated resistance. Front Plant Sci 11:37. https://doi.org/10.3389/fpls.2020.00037

Dellagi A, Segond D, Rigault M, Fagard M, Simon C, Saindrenan P, Expert D (2009) Microbial siderophores exert a subtle role in *Arabidopsis* during infection by manipulating the immune response and the iron status. Plant Physiol 150:1687–1696. https://doi.org/10.1104/pp.109.138636

Deshmukh SK, Gupta MK, Prakash V, Saxena S (2018) Endophytic fungi: a source of potential antifungal compounds. J Fungi 4:77. https://doi.org/10.3390/jof4030077

Djami-Tchatchou AT, Harrison GA, Harper CP, Wang R, Prigge MJ, Estelle M, Kunkel BN (2020) Dual role of auxin in regulating plant defense and bacterial virulence gene expression during *Pseudomonas syringae* PtoDC3000 pathogenesis. Mol Plant-Microbe Interact 33:8–1071. https://doi.org/10.1094/MPMI-02-20-0047-R

Dolatabad HK, Javan-Nikkhah M, Shier WT (2017) Evaluation of antifungal, phosphate solubilisation, and siderophore and chitinase release activities of endophytic fungi from *Pistacia vera*. Mycol Prog 16:777–790. https://doi.org/10.1007/s11557-017-1315-z

Faria DC, Dias ACF, Melo IS, de Carvalho Costa FE (2013) Endophytic bacteria isolated from orchid and their potential to promote plant growth. World J Microbiol Biotechnol 29:217–221. https://doi.org/10.1007/s11274-012-1173-4

Favre-Godal Q, Gourguillon L, Lordel-Madeleine S, Gindro K, Choisy P (2020) Orchids and their mycorrhizal fungi: an insufficiently explored relationship. Mycorrhiza 1–18 30:5–22. https://doi.org/10.1007/s00572-020-00934-2

Freitas-Mesquita AL, Meyer-Fernandes JR (2014) Biochemical properties and possible roles of ectophosphatase activities in fungi. Int J Mol Sci 15:2289–2304. https://doi.org/10.3390/ijms15022289

Fu S-F, Wei J-Y, Chen H-W, Liu YY, Lu HY, Chou JY (2015) Indole-3-acetic acid: a widespread physiological code in interactions of fungi with other organisms. Plant Signal Behav 10:e1048052. https://doi.org/10.1080/15592324.2015.1048052

Gianfreda L (2015) Enzymes of importance to rhizosphere processes. J Soil Sci Plant Nutr 15:283–306. https://doi.org/10.4067/S0718-95162015005000022

Givnish TJ, Spalink D, Ames M, Lyon SP, Hunter SJ, Zuluaga A, Iles WJD, Clements MA, Arroyo MTK, Leebens-Mack J, Endara L, Kriebel R, Neubig KM, Whitten WM, Williams NH, Cameron KM (2015) Orchid phylogenomics and multiple drivers of their extraordinary diversification. Proc Biol Sci 282:20151553. https://doi.org/10.1098/rspb.2015.1553

Glickmann E, Dessaux Y (1995) A critical examination of the specificity of the salkowski reagent for indolic compounds produced by phytopathogenic bacteria. Appl Environ Microbiol 61:793–796. https://doi.org/10.1128/AEM.61.2.793-796.1995

Gordon SA, Weber RP (1951) Colorimetric estimation of indoleacetic acid. Plant Physiol 26:192–195. https://doi.org/10.1104/pp.26.1.192

Goyal A, Kalia A (2020) Fungal phytohormones: plant growth-regulating substances and their applications in crop productivity. In: Yadav AN, Mishra S, Kour D et al (eds) Agriculturally important fungi for sustainable agriculture: Volume 1: Perspective for diversity and crop productivity. Springer International Publishing, Cham, pp 143–169

Gravel V, Antoun H, Tweddell RJ (2007) Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: possible role of indole acetic acid (IAA). Soil Biol Biochem 39:1968–1977. https://doi.org/10.1016/j.soilbio.2007.02.015

Guindon S, Dufayard J, Lefort V et al (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol 59:307–321. https://doi.org/10.1093/sysbio/syq010

Haas H, Eisendle M, Turgeon BG (2008) Siderophores in fungal physiology and virulence. Annu Rev Phytopathol 46:149–187. https://doi.org/10.1146/annurev.phyto.45.062806.094338

Hajieghrari B, Giglou MT (2008) Biological potantial of some Iranian *Trichoderma* isolates in the control of soil borne plant pathogenic fungi. Afr J Biotechnol 7:967–972. https://doi.org/10.4314/AJB.V7I8.58586

Hindt MN, Guerinot ML (2012) Getting a sense for signals: regulation of the plant iron deficiency response. Biochim Biophys Acta BBA - Mol Cell Res 1823:1521–1530. https://doi.org/10.1016/j.bbamcr.2012.03.010

Hiruma K (2019) Plant interactions with parasitic and beneficial *Colletotrichum* fungi under changing environmental conditions. J Gen Plant Pathol 85:468–470. https://doi.org/10.1007/s10327-019-00880-8

Hiruma K, Gerlach N, Sacristán S, Nakano RT, Hacquard S, Kracher B, Neumann U, Ramírez D, Bucher M, O'Connell RJ, Schulze-Lefert P (2016) Root endophyte *Colletotrichum tofieldiae* confers plant fitness benefits that are phosphate status dependent. Cell 165:464–474. https://doi.org/10.1016/j.cell.2016.02.028

Hofstetter V, Buyck B, Eyssartier G, Schnee S, Gindro K (2019) The unbearable lightness of sequenced-based identification. Fungal Divers 96:243–284. https://doi.org/10.1007/s13225-019-00428-3

Igiehon NO, Babalola OO (2018) Below-ground-above-ground plant-microbial interactions: focusing on soybean, rhizobacteria and mycorrhizal fungi. Open Microbiol J 12:261–279. https://doi.org/10.2174/1874285801812010261

Inglis PW, de CR PM, Resende LV, Grattapaglia D (2018) Fast and inexpensive protocols for consistent extraction of high quality DNA and RNA from challenging plant and fungal samples for high-throughput SNP genotyping and sequencing applications. PLoS One 13:e0206085. https://doi.org/10.1371/journal.pone.0206085

Jacquemyn H, Waud M, Lievens B, Brys R (2016) Differences in mycorrhizal communities between *Epipactis palustris, E. helleborine* and its presumed sister species *E. neerlandica*. Ann Bot 118:105–114. https://doi.org/10.1093/aob/mcw015

Jain A, Sarsaiya S, Chen J, Wu Q, Lu Y, Shi J (2021) Changes in global Orchidaceae disease geographical research trends: recent incidences, distributions, treatment, and challenges. Bioengineered 12:13–29. https://doi.org/10.1080/21655979.2020.1853447

Jain A, Sarsaiya S, Wu Q, Lu Y, Shi J (2019) A review of plant leaf fungal diseases and its environment speciation. Bioengineered 10:409–424. https://doi.org/10.1080/21655979.2019.1649520

Jia M, Chen L, Xin H-L, Zheng CJ, Rahman K, Han T, Qin LP (2016) A friendly relationship between endophytic fungi and medicinal plants: a systematic review. Front Microbiol 7:906. https://doi.org/10.3389/fmicb.2016.00906

Kadir NA, Naher L, Kayat F et al (2020) Morphological and molecular identification of *Fusarium* spp. and *Colletotrichum* spp. isolated from infected *Vanilla* orchid. Malays J Microbiol 17:42–51. https://doi.org/10.21161/mjm.200846

Kamel NM, Abdel-Motaal FF, El-Zayat SA (2020) Endophytic fungi from the medicinal herb *Euphorbia geniculata* as a potential source for bioactive metabolites. Arch Microbiol 202:247–255. https://doi.org/10.1007/s00203-019-01740-x

Khan AL, Halo BA, Elyassi A, Ali S, al-Hosni K, Hussain J, al-Harrasi A, Lee IJ (2016) Indole acetic acid and ACC deaminase from endophytic bacteria improves the growth of *Solanum lycopersicum*. Electron J Biotechnol 21:58–64. https://doi.org/10.1016/j.ejbt.2016.02.001

Khan RAA, Najeeb S, Hussain S, Xie B, Li Y (2020) Bioactive secondary metabolites from *Trichoderma* spp. against phytopathogenic fungi. Microorganisms 8:817. https://doi.org/10.3390/microorganisms8060817

King BC, Waxman KD, Nenni NV, Walker LP, Bergstrom GC, Gibson DM (2011) Arsenal of plant cell wall degrading enzymes reflects host preference among plant pathogenic fungi. Biotechnol Biofuels 4:4. https://doi.org/10.1186/1754-6834-4-4

Knapp DG, Kovács GM (2016) Interspecific metabolic diversity of root-colonizing endophytic fungi revealed by enzyme activity tests. FEMS Microbiol Ecol 92:12. https://doi.org/10.1093/femsec/fiw190

Kowalczyk A, Latowski D (2018) Role of plant-microorganism interactions in plant tolerance to arsenic. In: Hasanuzzaman M, Nahar K, Fujita M (eds) Mechanisms of arsenic toxicity and tolerance in plants. Springer, Singapore, pp 219–237

Kramer J, Özkaya Ö, Kümmerli R (2020) Bacterial siderophores in community and host interactions. Nat Rev Microbiol 18:152–163. https://doi.org/10.1038/s41579-019-0284-4

Kundan R, Pant G, Jadon N, Agrawal PK (2015) Plant growth promoting rhizobacteria: mechanism and current prospective. J Fertil Pestic 06:155. https://doi.org/10.4172/2471-2728.1000155

Kunkel BN, Harper CP (2018) The roles of auxin during interactions between bacterial plant pathogens and their hosts. J Exp Bot 69:245–254. https://doi.org/10.1093/jxb/erx447

Kuraku S, Zmasek CM, Nishimura O, Katoh K (2013) Leaves facilitates on-demand exploration of metazoan gene family trees on MAFFT sequence alignment server with enhanced interactivity. Nucleic Acids Res 41:W22–W28. https://doi.org/10.1093/nar/gkt389

Li Destri Nicosia MG, Mosca S, Mercurio R, Schena L (2014) Dieback of *Pinus nigra* seedlings caused by a strain of *Trichoderma viride*. Plant Dis 99:44–49. https://doi.org/10.1094/PDIS-04-14-0433-RE

Lionetti V, Métraux J-P (2014) Plant cell wall in pathogenesis, parasitism and symbiosis. Front Plant Sci 5:612. https://doi.org/10.3389/fpls.2014.00612

López-Millán A-F, Grusak MA, Abadía A, Abadía J (2013) Iron deficiency in plants: an insight from proteomic approaches. Front Plant Sci 4:75–86. https://doi.org/10.3389/fpls.2013.00254

Lynne AM, Haarmann D, Louden BC (2011) Use of blue agar CAS assay for siderophore detection. J Microbiol Biol Educ 12:51–53. https://doi.org/10.1128/jmbe.v12i1.249

Ma X, Nontachaiyapoom S, Jayawardena RS, Hyde KD, Gentekaki E, Zhou S, Qian Y, Wen T, Kang J (2018) Endophytic *Colletotrichum* species from *Dendrobium* spp. in China and northern Thailand. MycoKeys 43:23–57. https://doi.org/10.3897/mycokeys.43.25081

Maddison DR, Maddison WP (2000) Macclade 4: analysis of phylogeny and character evolution. Sinauer Associates Inc, Sunderland, MA

Mattoo AJ, Nonzom S (2021) Endophytic fungi: understanding complex cross-talks. Symbiosis 83:237–264. https://doi.org/10.1007/s13199-020-00744-2

Mayerhofer MS, Kernaghan G, Harper KA (2013) The effects of fungal root endophytes on plant growth: a meta-analysis. Mycorrhiza 23:119–128. https://doi.org/10.1007/s00572-012-0456-9

McMillian RTM Jr (2011) Efficacy of fungicides for control of *Colletotrichum gloeosporioides* on *Dendrobiums*. Proc Fla State Hortic Soc 124:314–316

Mehmood A, Hussain A, Irshad M, Hamayun M, Iqbal A, Khan N (2019) In vitro production of IAA by endophytic fungus *Aspergillus awamori* and its growth promoting activities in *Zea mays*. Symbiosis 77:225–235. https://doi.org/10.1007/s13199-018-0583-y

Meng Y-Y, Shao S-C, Liu S-J, Gao J-Y (2019) Do the fungi associated with roots of adult plants support seed germination? A case study on *Dendrobium exile* (Orchidaceae). Glob Ecol Conserv 17:e00582. https://doi.org/10.1016/j.gecco.2019.e00582

Milagres AMF, Machuca DNA (1999) Detection of siderophore production from several fungi and bacteria by a modification of chrome azurol S (CAS) agar plate assay. ScienceDirect J Microbiol Methods 37:1–6. https://doi.org/10.1016/S0167-7012(99)00028-7

Mostert L, Crous P, Petrini O (2000) Endophytic fungi associated with shoots and leaves of *Vitis vinifera*, with specific reference to the *Phomopsis viticola* complex. Sydowia 52:46–58. https://doi.org/10.3390/biology9120420

Munasinghe MVK, Kumar NS, Jayasinghe L, Fujimoto Y (2017) Indole-3-acetic acid production by *Colletotrichum siamense*, an endophytic fungus from *Piper nigrum* leaves. J Biol Act Prod Nat 7:475–479. https://doi.org/10.1080/22311866.2017.1408429

Ng TB, Liu J, Wong JH, Ye X, Wing Sze SC, Tong Y, Zhang KY (2012) Review of research on *Dendrobium*, a prized folk medicine. Appl Microbiol Biotechnol 93:1795–1803. https://doi.org/10.1007/s00253-011-3829-7

Novotná A, Benítez Á, Herrera P, Cruz D, Filipczyková E, Suárez JP (2018) High diversity of root-associated fungi isolated from three epiphytic orchids in southern Ecuador. Mycoscience 59:24–32. https://doi.org/10.1016/j.myc.2017.07.007

Nugraha AS, Triatmoko B, Wangchuk P, Keller PA (2020) Vascular epiphytic medicinal plants as sources of therapeutic agents: their ethnopharmacological uses, chemical composition, and biological activities. Biomolecules 10:181. https://doi.org/10.3390/biom10020181

Park M-J, Back C-G, Park J-H (2020) *Colletotrichum cymbidiicola* causing anthracnose on *Cymbidium* orchids in Korea. Mycobiology 48:321–325. https://doi.org/10.1080/12298093.2020.1768626

Parthibhan S, Rao MV, Senthil Kumar T (2017) Culturable fungal endophytes in shoots of *Dendrobium aqueum* Lindley – an imperiled orchid. Ecol Genet Genomics 3–5:18–24. https://doi.org/10.1016/j.egg.2017.06.004

Petrini O (1991) Fungal endophytes of tree leaves. In: Andrews JH, Hirano SS (eds) Microbial ecology of leaves. Springer, New York, pp 179–197

Phillips RD, Barrett MD, Dixon KW, Hopper SD (2011) Do mycorrhizal symbioses cause rarity in orchids? J Ecol 99:858–869. https://doi.org/10.1111/j.1365-2745.2011.01797.x

Ruddat M, Kokontis J, Birch L, Garber ED, Chiang KS, Campanella J, Dai H (1991) Interactions of *Microbotryum violaceum* (*Ustilago violacea*) with its host plant *Silene alba*. Plant Sci 80:157–165. https://doi.org/10.1016/0168-9452(91)90280-L

Saravanakumar K, Wang M-H (2020) Isolation and molecular identification of *Trichoderma* species from wetland soil and their antagonistic activity against phytopathogens. Physiol Mol Plant Pathol 109:101458. https://doi.org/10.1016/j.pmpp.2020.101458

Sarsaiya S, Jain A, Fan X, Jia Q, Xu Q, Shu F, Zhou Q, Shi J, Chen J (2020a) New insights into detection of a dendrobine compound from a novel endophytic *Trichoderma longibrachiatum* strain and its toxicity against phytopathogenic bacteria. Front Microbiol 11:337. https://doi.org/10.3389/fmicb.2020.00337

Sarsaiya S, Jain A, Jia Q, Fan X, Shu F, Chen Z, Zhou Q, Shi J, Chen J (2020b) Molecular identification of endophytic fungi and their pathogenicity evaluation against *Dendrobium nobile* and *Dendrobium officinale*. Int J Mol Sci 21:316. https://doi.org/10.3390/ijms21010316

Sarsaiya S, Jia Q, Fan X, Jain A, Shu F, Chen J, Lu Y, Shi J (2019a) First report of leaf black circular spots on *Dendrobium nobile* caused by *Trichoderma longibrachiatum* in Guizhou province, China. Plant Dis 103:3275. https://doi.org/10.1094/PDIS-03-19-0672-PDN

Sarsaiya S, Shi J, Chen J (2019b) A comprehensive review on fungal endophytes and its dynamics on Orchidaceae plants: current research, challenges, and future possibilities. Bioengineered 10:316–334. https://doi.org/10.1080/21655979.2019.1644854

Selosse M-A (2014) The latest news from biological interactions in orchids: in love, head to toe. New Phytol 202:337–340. https://doi.org/10.1111/nph.12769

Shah S, Shrestha R, Maharjan S, Selosse MA, Pant B (2019) Isolation and characterization of plant growth-promoting endophytic fungi from the roots of *Dendrobium moniliforme*. Plants 8:5. https://doi.org/10.3390/plants8010005

Shi Y, Xie H, Cao L, Zhang R, Xu Z, Wang Z, Deng Z (2017) Effects of cd- and Pb-resistant endophytic fungi on growth and phytoextraction of *Brassica napus* in metal-contaminated soils. Environ Sci Pollut Res 24:417–426. https://doi.org/10.1007/s11356-016-7693-y

Soelistijono R, Utami DS, Daryanti et al (2020) Short communication: characterization of rhizoctonia-like mycorrhizae associated with five *Dendrobium* species in Java, Indonesia. Biodiversitas J Biol Divers 21:1007–1011. https://doi.org/10.13057/biodiv/d210321

Sood M, Kapoor D, Kumar V, Sheteiwy MS, Ramakrishnan M, Landi M, Araniti F, Sharma A (2020) *Trichoderma:* the "secrets" of a multitalented biocontrol agent. Plants Basel Switz 9:762. https://doi.org/10.3390/plants9060762

Spaepen S, Vanderleyden J (2011) Auxin and plant-microbe interactions. Cold Spring Harb Perspect Biol 3:a001438. https://doi.org/10.1101/cshperspect.a001438

Srivastava S, Kadooka C, Uchida JY (2018) *Fusarium* species as pathogen on orchids. Microbiol Res 207:188–195. https://doi.org/10.1016/j.micres.2017.12.002

Streletskii RA, Kachalkin AV, Glushakova AM, Demin VV, Chernov IY (2016) Quantitative determination of indole-3-acetic acid in yeasts using high performance liquid chromatography—tandem mass spectrometry. Microbiology 85:727–736. https://doi.org/10.1134/S0026261716060187

Stringlis IA, Zhang H, Pieterse CMJ, Bolton MD, de Jonge R (2018) Microbial small molecules - weapons of plant subversion. Nat Prod Rep 35:410–433. https://doi.org/10.1039/c7np00062f

Taghinasab M, Jabaji S (2020) Cannabis microbiome and the role of endophytes in modulating the production of secondary metabolites: an overview. Microorganisms 8:355. https://doi.org/10.3390/microorganisms8030355

Taguchi F, Suzuki T, Inagaki Y, Toyoda K, Shiraishi T, Ichinose Y (2010) The siderophore pyoverdine of *Pseudomonas syringae* pv. Tabaci 6605 is an intrinsic virulence factor in host tobacco infection. J Bacteriol 192:117–126. https://doi.org/10.1128/JB.00689-09

Teixeira da Silva JA, Tsavkelova EA, Zeng S, Ng TB, Parthibhan S, Dobránszki J, Cardoso JC, Rao MV (2015) Symbiotic in vitro seed propagation of *Dendrobium*: fungal and bacterial partners and their influence on plant growth and development. Planta 242:1–22. https://doi.org/10.1007/s00425-015-2301-9

Tejesvi MV, Pirttilä AM (2018) Endophytic fungi, occurrence, and metabolites. In: Anke T, Schüffler A (eds) Physiology and genetics: selected basic and applied aspects. Springer International Publishing, Cham, pp 213–230

Tekere M, Mswaka AY, Zvauya R, Read JS (2001) Growth, dye degradation and ligninolytic activity studies on Zimbabwean white rot fungi. Enzym Microb Technol 28:420–426. https://doi.org/10.1016/S0141-0229(00)00343-4

Tian Y, Amand S, Buisson D, Kunz C, Hachette F, Dupont J, Nay B, Prado S (2014) The fungal leaf endophyte *Paraconiothyrium variabile* specifically metabolizes the host-plant metabolome for its own benefit. Phytochemistry 108:95–101. https://doi.org/10.1016/j.phytochem.2014.09.021

Trivedi P, Leach JE, Tringe SG, Sa T, Singh BK (2020) Plant–microbiome interactions: from community assembly to plant health. Nat Rev Microbiol 18:607–621. https://doi.org/10.1038/s41579-020-0412-1

Vinale F, Flematti G, Sivasithamparam K, Lorito M, Marra R, Skelton BW, Ghisalberti EL (2009) Harzianic acid, an antifungal and plant growth promoting metabolite from *Trichoderma harzianum*. J Nat Prod 72:2032–2035. https://doi.org/10.1021/np900548p

Vinale F, Nigro M, Sivasithamparam K, Flematti G, Ghisalberti EL, Ruocco M, Varlese R, Marra R, Lanzuise S, Eid A, Woo SL, Lorito M (2013) Harzianic acid: a novel siderophore from *Trichoderma harzianum*. FEMS Microbiol Lett 347:123–129. https://doi.org/10.1111/1574-6968.12231

Wang Y-H (2021) Traditional uses and pharmacologically active constituents of *Dendrobium* plants for dermatological disorders: a review. Nat Prod Bioprospecting. https://doi.org/10.1007/s13659-021-00305-0

Xing X, Ma X, Deng Z, Chen J, Wu F, Guo S (2013) Specificity and preference of mycorrhizal associations in two species of the genus *Dendrobium* (Orchidaceae). Mycorrhiza 23:317–324. https://doi.org/10.1007/s00572-012-0473-8

Xing Y-M, Chen J, Cui J-L, Chen XM, Guo SX (2011) Antimicrobial activity and biodiversity of endophytic fungi in *Dendrobium devonianum* and *Dendrobium thyrsiflorum* from Vietman. Curr Microbiol 62:1218–1224. https://doi.org/10.1007/s00284-010-9848-2

XiQing Z, JiChuan K, Jin H et al (2008) Study on identification of two strains of endophytic *Xylaria* of *Dendrobium* and its active composition. Southwest China J Agric Sci 21:317–322

Yamaji K, Watanabe Y, Masuya H, Shigeto A, Yui H, Haruma T (2016) Root fungal endophytes enhance heavy-metal stress tolerance of *Clethra barbinervis* growing naturally at mining sites via growth enhancement, promotion of nutrient uptake and decrease of heavy-metal concentration. PLoS One 11:1–15. https://doi.org/10.1371/journal.pone.0169089

Yan L, Zhu J, Zhao X, Shi J, Jiang C, Shao D (2019) Beneficial effects of endophytic fungi colonization on plants. Appl Microbiol Biotechnol 103:3327–3340. https://doi.org/10.1007/s00253-019-09713-2

Yang G, Hu Y, Fasoyin OE, Yue Y, Chen L, Qiu Y, Wang X, Zhuang Z, Wang S (2018) The *Aspergillus flavus* phosphatase CDC14 regulates development, aflatoxin biosynthesis and pathogenicity. Front Cell Infect Microbiol 8:141. https://doi.org/10.3389/fcimb.2018.00141

Yao H, Sun X, He C, Maitra P, Li XC, Guo LD (2019) Phyllosphere epiphytic and endophytic fungal community and network structures differ in a tropical mangrove ecosystem. Microbiome 7:57. https://doi.org/10.1186/s40168-019-0671-0

Yin C, Park J-J, Gang DR, Hulbert SH (2014) Characterization of a tryptophan 2-monooxygenase gene from *Puccinia graminis f.* sp. *tritici* involved in auxin biosynthesis and rust pathogenicity. Mol Plant-Microbe Interact 27:227–235. https://doi.org/10.1094/MPMI-09-13-0289-FI

Zhang F, Lv Y, Zhao Y, Guo S (2013) Promoting role of an endophyte on the growth and contents of kinsenosides and flavonoids of *Anoectochilus formosanus* Hayata, a rare and threatened medicinal Orchidaceae plant. J Zhejiang Univ Sci B 14:785–792. https://doi.org/10.1631/jzus.B1300056

Żukiewicz-Sobczak WA, Cholewa G, Sobczak P, Silny W, Nadulski R, Wojtyła-Buciora P, Zagórski J (2016) Enzymatic activity of fungi isolated from crops. Postepy Dermatol Alergol 33:457–463. https://doi.org/10.5114/ada.2016.63885