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Secondary metabolites from the endophytic fungus *Talaromyces pinophilus*

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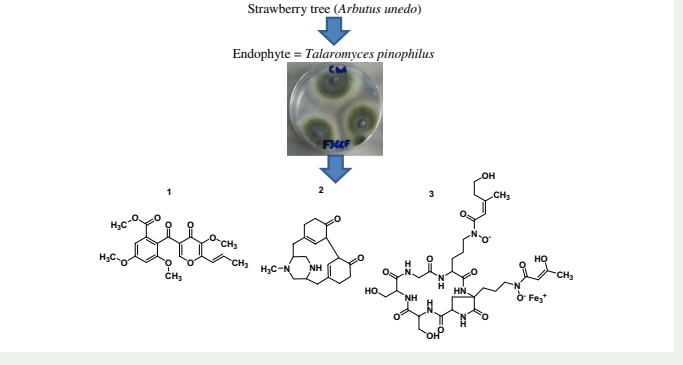
ABSTRACT

Endophytic fungi have a great influence on plant health and growth, and are an important source of bioactive natural compounds. Organic extracts obtained from the culture filtrate of an endophytic strain of *Talaromyces pinophilus* isolated from strawberry tree (*Arbutus unedo*) were studied. Metabolomic analysis revealed the presence of three bioactive metabolites, the siderophore ferrirubin, the platelet-aggregation inhibitor herquline B and the antibiotic 3-O-methylfunicone. The latter was the major metabolite produced by this strain and displayed toxic effects against the pea aphid *Acyrthosiphon pisum* (Homoptera Aphidiidae). This toxicity represents an additional indication that the widespread endophytic occurrence of *T. pinophilus* may be related to a possible role in defensive mutualism. Moreover, the toxic activity on aphids could promote further study on 3-O-methylfunicone, or its derivatives, as an alternative to synthetic chemicals in agriculture.



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1. Introduction

Plants live in association with micro-organisms with different levels of interaction. This assumption stimulates insights on plant microbiome, intended as the collective genome of micro-organisms living in contact with plants (Schlaeppi & Bulgarelli 2015), and new concepts in plant evolution have been developed considering a basic role of the associated fungal endophytes (Hardoim et al. 2015). Regarded as an under-explored niche of chemodiversity (Kusari et al. 2014), endophytic fungi have a recognised ability to produce bioactive compounds which may play a role in plant protection against pathogens and pests (Nicoletti & Fiorentino 2015; Gouda et al. 2016).

Colonisation by endophytes may offer significant benefits to their host plants by producing various metabolites that protect against pathogen attack, promote plant (or vegetative) growth, improve crop yields, show herbicide activity and induce resistance. Fungal natural products are currently used in agriculture as active ingredients of different bioformulates (Copping & Duke 2007), and several endophytes are known to have anti-insect properties (Barelli et al. 2016). Although bioinsecticides currently occupy only a small amount of the market, these compounds are very interesting and their use is constantly increasing (Guo et al. 2008).

Endophytic strains of fungi belonging to the genus *Talaromyces* have attracted considerable attention due to their ability to produce a variety of interesting bioactive compounds. Examples of secondary metabolites produced by *Talaromyces* species include tetraene lactones, diphenyl ether derivatives, epiaustdiol, furanosteroid and anthraquinones (Xie et al. 2016; Zhai et al. 2016; Zhi et al. 2016). The endophytic behaviour seems to represent an ecological connotation of several *Talaromyces* species (Li et al. 2010; Bara et al. 2013). Recent studies isolated and characterised novel bioactive compounds produced by *Talaromyces* spp. (Li et al. 2010; Bara et al. 2013) that may have positive implications in crop protection (Kim et al. 2007).

Our work focused on the isolation of bioactive secondary metabolites produced by endophytic fungus *Talaromyces pinophilus* strain F36CF in liquid cultures. The presence of other secondary metabolites released in the culture filtrate has been also investigated by metabolomic analysis. In addition, we report the insecticidal activity on aphids both the organic extracts obtained by strain F36CF and its main secondary metabolite 3-O-methylfunicone.

2. Results and discussion

Strain F36CF of *T. pinophilus* was isolated from a strawberry tree (*Arbutus unedo*) by following a standard protocol (Nicoletti et al. 2013). Fungal strain identification was performed according to morphological and molecular analyses, as previously reported in literature (Yilmaz et al. 2014). The alignment of the ITS sequences of F36CF (KX640963) and the type strain of *T. pinophilus* CBS 631.66 (JN899382) gave an identity of 98.5% (Figure S1A). In addition, a phylogenetic tree based on β-tubulin (*benA*) gene sequence was constructed to confirm the relationships among *Talaromyces* species and *T. pinophilus* strain F36CF (Figure S1B). F36CF clearly clustered with *T. pinophilus* strains, while the closest relative species (*T. liani* and *T. sayulitensis*) formed two distinct groups (*T. dendriticus* was chosen as an out-group).

The ethyl acetate crude extract of F36CF culture filtrate was fractionated by column chromatography and silica-gel TLC to obtain as main compound a metabolite that showed the

NMR signals reported by De Stefano et al. (1999) for (E)-3-methoxy-2-propenyl-5-(2′-carbomethoxy-4′-6′-dimethoxybenzoyl)-4-pyrone named 3-O-methylfunicone (OMF). The identification of OMF was also confirmed by LC-MS/MS qTOF analysis. The metabolite was detected at 10.6 min (Figure S21A) and mass spectra analysis confirmed the presence of the molecular ion $[M - H]^+$ at $m/z = 389.1232$ (Figure S2B), corresponding to the molecular formula $C_{20}H_{20}O_8$ (Calcd 388.1236); other signals were also detected at 799.2203 $[M_2 + Na]^+$; 427.0782 $[M + K]^+$; 411.1049 $[M + Na]^+$; 357.0969 $[M - CO]^+$ (Figure S2B). OMF was not present in the mycelia organic extract that mainly revealed the presence of fatty acids.

The production of bioactive metabolites by F36CF was also investigated by LC–MS approach. The metabolomic analysis of the F36CF culture filtrates showed the presence of three known molecules, herquline B, ferrirubin and OMF (Figure 1). OMF was found to be the most abundant secondary metabolite biosynthesised by F36CF, yielding 5.5 mg of pure compound per litre of culture filtrate.

The other two compounds identified in the present study, herquline B and ferrirubin, were detected in *Talaromyces* for the first time and integrate the known chemotaxonomic profile of *T. pinophilus*. These metabolites were confidently identified by comparing the LC–MS qTOF data with known compounds present in an in-house database including over 4000 fungal secondary metabolites. In particular, both herquline B and ferrirubin yielded a score matching ≥95% (Figure S3).

Interestingly herquline B was previously isolated only from a soil strain of *Penicillium herquei,* and has been characterised as a platelet anti-aggregation factor but showed no antibiotic activity (Enomoto et al. 1996). Conversely, ferrirubin is a ferrichrome-type siderophore reported from many filamentous fungi (Heymann et al. 2000). Besides its primary function related to iron transportation, this class of compounds has also shown a plant growth promoting effect and antibiotic activity, thus suggesting involvement in the plant-fungus endophytic relationship (Bartholdy et al. 2001; Ahmed & Holmström 2014).

The F36C crude extract and OMF were tested on broad bean (*Vicia faba*) leaves against the pea aphid *Acyrthosiphon pisum* (Homoptera Aphidiidae), and demonstrated a mortality (according to Abbott's formula) of 35.7% and 26.2% at 72 h after treatment (Table 1). We observed that the contact activity increased over the time, particularly in the case of the total extract as compared to OMF. This result is typical of insecticides based on natural extracts, whose bioactivity may be related to a single compound or derives from the synergism between different molecules (De Feo et al. 2009).

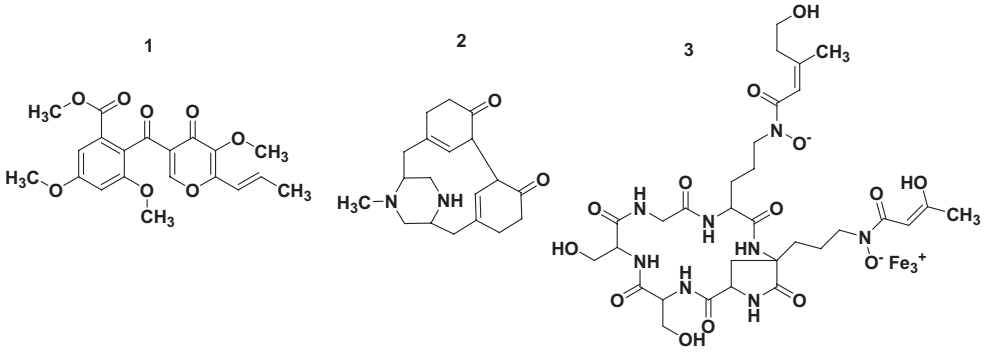

Figure 1. Chemical structures of 3-O-methylfunicone (**1**), herquline B (**2**) and ferrirubin (**3**).

Table 1. Evaluation of insecticidal activity by *T. pinophilus* extract and OMF as determined by using Abbott's formula to compensate for mortality caused by the control solvent (Rosenheim & Hoy 1989).

Hours after treatment	Abbott value (%) of OMF	Abbott value (%) of *T. pinophilus* extract
24	14.8	13.0
48	11.3	9.4
72	26.2	35.7

Table 2. Endophytic occurrence of *T. pinophilus* on diverse host plants.

Host plant	Location	Reference
Drosera rotundifolia	Snowdonia National Park (Great Britain)	Quilliam & Jones 2010
Osbeckia chinensis	Meghalaya (India)	Bhagobaty & Joshi 2011
Ceriops tagal	DongZhai Gang, Hainan (China)	Xing & Guo 2011
Laguncularia racemosa	Itamaracá Island, Pernambuco (Brazil)	Costa et al. 2012
Gloriosa superba	Patalkot valley, Madhya Pradesh (India)	Budhiraja et al. 2012
Carex scabrifolia	Suncheon Bay (South Korea)	You et al. 2012
Suaeda japonica		
Artemisia fukudo	Ulleung Island (South Korea)	Kim et al. 2012
Setaria viridis		
Asparagus schoberioides	Dokdo Islands (South Korea)	You et al. 2013
Setaria viridis		
Limonium tetragonum	Muan (South Korea)	Khalmuratova et al. 2015
Suaeda glauca		
Allium fistulosum	Lanzhou (China)	Zhai et al. 2015
Allium schoenoprasum	Nattha Top, Kashmir (India)	Koul et al. 2016
Hypericum mysorense	Talacauvery, Karnataka (India)	Samaga & Rai 2016
Curcuma amada	Karnataka (India)	Krishnapura & Belur 2016

OMF is a 4-pyrone derivative belonging to a class of fungal compounds mainly isolated from species of *Talaromyces* (Nicoletti et al. 2009), which exhibited notable fungitoxic and antitumour properties (De Stefano et al. 1999; Nicoletti et al. 2014). The observed insecticidal effect expands the range of bioactivities associated with this molecule, stimulating further insights on its biomolecular mechanisms of action. However, our results support the hypothesis that the mutualistic relationship established by some endophytic fungi with their host plant involves the production of microbial compounds able to provide protection against pests and pathogens. In this regard, recent reports from different ecological contexts worldwide have disclosed a widespread endophytic adaptation of *T. pinophilus* (= *Penicillium pinophilum*) (Table 2). This fungus has been involved in different types of interactions with plants and their associated pests and pathogens, and opens novel opportunities for research on biological control agents and related bioactive metabolites (Schulz et al. 2015; Barelli et al. 2016; Keyser et al. 2016).

3. Experimental

3.1. General considerations

All LC–MS analyses were done on an Agilent HP 1260 Infinity Series liquid chromatograph with a DAD system (Agilent Technologies, Torrance, CA, USA) coupled to a Q-TOF mass spectrometer model G6540B (Agilent Technologies) with a Dual ESI source. Separations were performed on a Zorbax Eclipse Plus C18 column, 4.6 × 100 mm, with 3.5 mm particles (Agilent Technologies), operating at a constant temperature of 37°C. The analyses were done at a flow-rate of 0.6 mL/min with a water (H_2O) – acetonitrile (ACN) gradient, starting at ACN

– H_2O (5:95) going to 100% ACN in 12 min, maintaining 100% ACN for 3 min, before returning to the start conditions in 2 min and equilibrating for 3 min. Both solvents were acidified with 0.1% formic acid. The UV spectra were collected by DAD every 0.4 s from 190 to 750 nm with a resolution of 2 nm. The MS system operated in the positive ESI mode and TOF-MS scanning was collected as centroid from m/z 100 to 1600 for all samples at a scan rate of 3 spectra/s. To perform the real-time lock mass correction, two reference mass compounds, a lock mass solution including purine ($C_5H_4N_4$ at *m/z* 121.050873, 10 µmol L^{-1}) and hexakis (1H,1H, 3H-tetrafluoropentoxy)-phosphazene ($C_{18}H_{18}O_6N_3P_3F_{24}$ at *m/z* 922.009798, 2 µmol L^{-1}) were used. The capillary was held at 4000 V, fragmentor at 180 V, cone 1 (skimmer 1) at 45 V. Gas temperature was 350°C during the run at 11 L/min, and the nebuliser was set at 45 psig. The injected sample volume was 5 µL. Solvents were LC–MS grade, and all other chemicals were analytical grade. All were from Sigma-Aldrich (Germany) unless otherwise stated. ESI–TOF tune mix was purchased from Agilent Technologies (Torrance, CA, USA). The chromatograms were analysed by Mass Hunter software (Agilent Technologies) matching their data with known compounds registered in an in-house database including over 4000 fungal secondary metabolites.

^1H and ^{13}C NMR spectra were recorded with a 400 MHz Bruker Avance spectrometer, equipped with a 5 mm Bruker Broad Band Inverse probe (BBI), working at the ^1H and ^{13}C frequencies of 400.13 and 100.61 MHz, respectively. Peak of chloroform was used as reference to calibrate both ^1H and ^{13}C axes.

Column chromatography was performed on silica gel (Merck silica gel 60 GF254), and TLC with glass pre-coated silica gel GF254 plates (Merck Kieselgel 60 GF254, 0.25 mm). The compounds were detected on TLC plates using UV light (254 or 366 nm), and/or by spraying the plates with 5% (v/v) H_2SO_4 solution in EtOH followed by heating at 110°C for 10 min.

3.2. Fungal strain isolation and identification

Strain F36CF was recovered from a secondary branch of a strawberry tree collected in the isle of Favignana, off the coast of western Sicily (37.921545 N, 12.32123 E). The sampled cutting was processed according to a standard protocol for isolation of endophytic fungi (Nicoletti et al. 2013). The fungus was identified according to morphological characters and molecular analyses rDNA-ITS and β-tubulin gene sequence (Yilmaz et al. 2014).

F36CF was inoculated on Potato Dextose Agar (PDA, Hi-Media, India) plates and cultivated for 10 days at 25°C. Fungal mycelium was collected and genomic DNA was isolated using NucleoSpin® Soil kit (Macherey-Nagel, Düren, Germany), following manufacturer's instructions. DNA quantity was determined using a Qubit 2.0 fluorometer with the dsDNA BR assay (Life Technologies, Grand Island, NY). PCR analysis was carried out in 50 µl total reaction volume, with 0.5 µM primer, 0.2 mM dNTP Mix, 1 × DreamTaq Green Buffer (Thermo scientific), and 1.25 U of DreamTaq DNA Polymerase (Thermo scientific). The internal transcribed spacer region (ITS) was amplified using the primer pair ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). β-tubulin (*benA*) gene was amplified using the primer pair Bt2a (5′-GGTAACCAAATCGGTGCTGCTTTC-3′) and Bt2b (5′-ACCCTCAGTGTAGTGACCC TTGGC-3′). Sequencing reactions were performed by Eurofins Genomics with the same primer sets used for PCR amplification. Sequences alignments were carried out through Clustal Omega multiple sequence alignment program tool of the EMBL-EBI web site (https://www.ebi.ac.uk/Tools/msa/clustalo). Molecular phylogenetic analysis by Maximum Likelihood method for *benA* gene was conducted using MEGA7 software (www.megasoftware.net).

Sequences have been deposited in GenBank under the reference numbers KX640963 (ITS) and KX985415 (benA).

3.3. Extraction and isolation

F36CF mycelial plugs from actively growing cultures were used to inoculate 150 mL potato dextrose broth (PDB, HI-MEDIA) in 500 mL Erlenmayer flask (10 conical flasks). The stationary culture was kept incubated in the dark at 26 ± 2°C for 21 days. The fungal cultures were vacuum filtered through filter paper (Whatman No. 4) to remove the biomass, and the culture filtrate was collected and then concentrated 10-times by lyophilization. The culture filtrate was extracted exhaustively with ethyl acetate and the combined organic extracts were dried (Na_2SO_4) and evaporated under reduced pressure at 37°C.

The crude extract (120 mg) was fractionated by column chromatography isocratically eluted with CH_2Cl_2–MeOH (98:2, v:v). Seven fractions were collected and pooled on the basis of similar TLC profiles. Fraction 2 was further purified by silica-gel preparative TLC (CH_2Cl_2–MeOH 98:2 v:v) to yield 8 mg of OMF (**1** in Figure 1).

3.4. Bioassays against aphids

Insecticidal activity was tested against the pea aphid *A. pisum*. Twenty 3rd-instar nymphs, obtained from a synchronised parthenogenetic population reared on broad bean (*Vicia faba*) plants were placed on two circular (35 mm diameter) leaf disks laid upside down on 2% (w/v) water agar in a 90 mm diameter petri dish. One mL of the test solution was sprayed onto the aphids, and the treated insects were incubated at 20–22°C and 16:8 h (light/dark) photoperiod. The number of dead aphids was assessed 24, 48 and 72 h after treatment. The classical Abbott's formula (Rosenheim & Hoy 1989) was used to correct bioassay data for control response (solvent-treated aphids) and compute the insecticidal effectiveness of a compound. In bioassays OMF was tested at 50 µg mL^{-1}, prepared by dissolving the purified compound in 4 µL of dichloromethane and adjusting the concentration with distilled water.

4. Conclusion

According to its metabolomic profile and the emerging widespread endophytic behaviour, the strain F36CF of *T. pinophilus,* identified in this work, represents an interesting example of a micro-organism with a multifunctional lifestyle. The production of secondary metabolites is important for the chemotaxonomic characterisation of F36CF isolate. In addition, a novel insecticidal activity of both F36CF organic extracts and its main metabolite OMF on aphids has been demonstrated. Our results may help the development of new biopesticide formulations based on bioactive metabolites and further studies are necessary for future applications in agriculture.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Ahmed E, Holmström SJ. 2014. Siderophores in environmental research: roles and applications. Microb Biotechnol. 7:196–208.

Bara R, Aly AH, Wray V, Lin W, Proksch P, Debbab A. 2013. Talaromins A and B, new cyclic peptides from the endophytic fungus *Talaromyces wortmannii*. Tetrahedron Lett. 54:1686–1689.

Barelli L, Moonjely S, Behie SW, Bidochka MJ. 2016. Fungi with multifunctional lifestyles: endophytic insect pathogenic fungi. Plant Mol Biol. 90:657–664.

Bartholdy BA, Berreck M, Haselwandter K. 2001. Hydroxamate siderophore synthesis by *Phialocephala fortinii*, a typical dark septate fungal root endophyte. BioMetals. 14:33–42.

Bhagobaty RK, Joshi SR. 2011. Multi-loci molecular characterisation of endophytic fungi isolated from five medicinal plants of Meghalaya, India. Mycobiology. 39:71–78.

Budhiraja A, Nepali K, Kaul S, Dhar KL. 2012. Antimicrobial and cytotoxic activities of fungal isolates of medicinal plant *Gloriosa superba*. Int J Recent Adv Pharm Res. 2:1–9.

Copping LG, Duke SO. 2007. Natural products that have been used commercially as crop protection agents. Pest Manag Sci. 63:524–554.

Costa IP, Maia LC, Cavalcanti MA. 2012. Diversity of leaf endophytic fungi in mangrove plants of Northeast Brazil. Braz J Microbiol. 43:1165–1173.

De Feo V, Mancini E, Voto E, Curini M, Digilio MC. 2009. Bioassay-oriented isolation of an insecticide from *Ailanthus altissima*. J Plant Interact. 4:119–123.

De Stefano S, Nicoletti R, Milone A, Zambardino S. 1999. 3-O-Methylfunicone, a fungitoxic metabolite produced by the fungus *Penicillium pinophilum*. Phytochemistry. 52:1399–1401.

Enomoto Y, Shiomi K, Hayashi M, Masuma R, Kawakubo T, Tomosawa K, Iwai Y, Omura S. 1996. Herquline B, a new platelet aggregation inhibitor produced by *Penicillium herquei* Fg-372. J Antib. 49:50–53.

Gouda S, Das G, Sen SK, Shin HS, Patra JK. 2016. Endophytes: a treasure house of bioactive compounds of medicinal importance. Frontiers Microbiol. 7:1538. doi:10.3389/fmicb.2016.01538.

Guo B, Wang Y, Sun X, Tang K. 2008. Bioactive natural products from endophytes: a review. Appl Biochem Microbiol. 44:136–142.

Hardoim PR, van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A, Döring M, Sessitsch A. 2015. The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol Mol Biol Rev. 79:293–320.

Heymann P, Ernst JF, Winkelmann G. 2000. Identification and substrate specificity of a ferrichrome-type siderophore transporter (Arn1p) in *Saccharomyces cerevisiae*. FEMS Microbiol Lett. 186:221–227.

Keyser CA, Jensen B, Meyling NV. 2016. Dual effects of Metarhizium spp. and Clonostachys rosea against an insect and a seed-borne pathogen in wheat. Pest Manag Sci. 72:517–526.

Khalmuratova I, Kim H, Nam YJ, Oh Y, Jeong MJ, Choi HR, You YH, Choo YS, Lee IJ, Shin JH, et al. 2015. Diversity and plant growth promoting capacity of endophytic fungi associated with halophytic plants from the West coast of Korea. Mycobiology. 43:373–383.

Kim HY, Choi GJ, Lee HB, Lee SW, Lim HK, Jang KS, Son SW, Lee SO, Cho KY, Sung ND, Kim JC. 2007. Some fungal endophytes from vegetable crops and their anti-oomycete activities against tomato late blight. Lett Appl Microbiol. 44:332–337.

Kim M, You YH, Yoon H, Kim H, Seo Y, Khalmuratova I, Shin JH, Lee IJ, Choo YS, Kim JG. 2012. Genetic diversity of endophytic fungal strains isolated from the roots of coastal plants in Ulleung island for restoration of coastal ecosystem. J Life Sci. 22:1384–1391.

Koul M, Meena S, Kumar A, Sharma PR, Singamaneni V, Riyaz-Ul-Hassan S, Hamid A, Chaubey A, Prabhakar A, Gupta P, Singh S. 2016. Secondary metabolites from endophytic fungus *Penicillium pinophilum* induce ROS-mediated apoptosis through mitochondrial pathway in pancreatic cancer cells. Planta Med. 82:344–355.

Krishnapura PR, Belur PD. 2016. Isolation and screening of endophytes from the rhizomes of some Zingiberaceae plants for L-asparaginase production. Prepar Biochem Biotechnol. 46:281–287.
Kusari S, Singh S, Jayabaskaran C. 2014. Biotechnological potential of plant-associated endophytic fungi: hope versus hype. Trends Biotechnol. 32:297–303.
Li LQ, Yang YG, Zeng Y, Zou C, Zhao PJ. 2010. A new azaphilone, kasanosin C, from an endophytic *Talaromyces* sp. T1BF. Molecules. 15:3993–3997.
Nicoletti R, Fiorentino A. 2015. Plant bioactive metabolites and drugs produced by endophytic fungi of Spermatophyta. Agriculture. 5:918–970.
Nicoletti R, Manzo E, Ciavatta ML. 2009. Occurence and bioactivities of funicone-related compounds. Int J Mol Sci. 10:1430–1444.
Nicoletti R, De Filippis A, Buommino E. 2013. Antagonistic aptitude and antiproliferative properties on tumor cells of fungal endophytes from the Astroni Nature Reserve, Italy. Afr J Microbiol Res. 7:4073–4083.
Nicoletti R, Scognamiglio M, Fiorentino A. 2014. Structural and bioactive properties of 3-O-methylfunicone. Mini Rev Med Chem. 14:1043–1047.
Quilliam RS, Jones DL. 2010. Fungal root endophytes of the carnivorous plant *Drosera rotundifolia*. Mycorrhiza. 20:341–348.
Rosenheim JA, Hoy MA. 1989. Confidence intervals for the Abbott's formula correction of bioassay data for control response. J Econ Entomol. 82:331–335.
Samaga PV, Rai VR. 2016. Diversity and bioactive potential of endophytic fungi from *Nothapodytes foetida*, *Hypericum mysorense* and *Hypericum japonicum* collected from Western Ghats of India. Ann Microbiol. 66:229–244.
Schlaeppi K, Bulgarelli D. 2015. The plant microbiome at work. Mol Plant-Microbe Interact. 28:212–217.
Schulz B, Haas S, Junker C, Andrée N, Schobert M. 2015. Fungal endophytes are involved in multiple balanced antagonisms. Curr Sci. 109:39–45.
Xie XS, Fang XW, Huang R, Zhang SP, Wei HX, Wu SH. 2016. A new dimeric anthraquinone from endophytic *Talaromyces* sp. YE3016. Nat Prod Res. 30:1–6.
Xing X, Guo S. 2011. Fungal endophyte communities in four Rhizophoraceae mangrove species on the south coast of China. Ecol Res. 26:403–409.
Yilmaz N, Visagie CM, Houbraken J, Frisvad JC, Samson RA. 2014. Polyphasic taxonomy of the genus *Talaromyces*. Stud Mycol. 78:175–341.
You YH, Yoon H, Kang SM, Shin JH, Choo YS, Lee IJ, Lee JM, Kim JG. 2012. Fungal diversity and plant growth promotion of endophytic fungi from six halophytes in Suncheon Bay. J Microbiol Biotechnol. 22:1549–1556.
You YH, Yoon H, Kim H, Lim SH, Shin JH, Lee IJ, Choo YS, Kim JG. 2013. Plant growth-promoting activity and genetic diversity of endophytic fungi isolated from native plants in Dokdo Islands for restoration of a coastal ecosystem. J Life Sci. 23:95–101.
Zhai MM, Niu HT, Li J, Xiao H, Shi YP, Di DL, Crews P, Wu QX. 2015. Talaromycolides A–C, novel phenyl-substituted phthalides isolated from the green Chinese onion-derived fungus *Talaromyces pinophilus* AF-02. J Agric Food Chem. 63:9558–9564.
Zhai MM, Li J, Jiang CX, Shi YP, Di DL, Crews P, Wu QX. 2016. The bioactive secondary metabolites from *Talaromyces* species. Nat Prod and Bioprosp. 6:1–24.
Zhi KK, Yang ZD, Zhou SY, Yao XJ, Li S, Zhang F. 2016. A new furanosteroid from *Talaromyces* sp. lgt-4, a fungal endophyte isolated from *Tripterygium wilfordii*. Nat Prod Res. 1:1–5.