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ORIGINAL ARTICLE

Metabolite analysis of endophytic fungi from cultivars of *Zingiber officinale* Rosc. identifies myriad of bioactive compounds including tyrosol

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Abstract Endophytic fungi associated with rhizomes of four cultivars of *Zingiber officinale* were identified by molecular and morphological methods and evaluated for their activity against soft rot pathogen *Pythium myriotylum* and clinical pathogens. The volatile bioactive metabolites produced by these isolates were identified by GC–MS analysis of the fungal crude extracts. Understanding of the metabolites produced by endophytes is also important in the context of raw consumption of ginger as medicine and spice. A total of fifteen isolates were identified from the four varieties studied. The various genera identified were *Acremonium* sp., *Gliocladiopsis* sp., *Fusarium* sp., *Colletotrichum* sp., *Aspergillus* sp., *Phlebia* sp., *Earliella* sp., and *Pseudolagarobasidium* sp. The endophytic community was unique to each variety, which could be due to the varying host genotype. Fungi from phylum Basidiomycota were identified for the first time from ginger. Seven isolates showed activity against *Pythium*, while only two showed antibacterial activity. The bioactive metabolites identified in the fungal crude extracts include tyrosol, benzene acetic acid, ergone, dehydromevalonic lactone, *N*-aminopyrrolidine, and many bioactive fatty acids and their derivatives which included linoleic acid, oleic acid, myristic acid, *n*-hexadecanoic acid, palmitic acid methyl ester, and methyl linoleate. The presence of these varying bioactive endophytic fungi may be one of the reasons for the differences in the performance of the different ginger varieties.

Keywords Ginger · Endophytic fungi · GC–MS · Metabolite analysis · Tyrosol

Introduction

Zingiber officinale or ginger is an outstanding member among the 1400 species in the family Zingiberaceae (Le et al. 2014). Due to its unique flavor, it is a favorite spice across the globe. Ginger has been widely used in Ayurvedic, Chinese, and Tibb-Unani herbal formulations, since ancient times for treatment of unrelated ailments including indigestion, vomiting, constipation, dementia, hypertension, arthritis, rheumatism, pains, sprains, sore throat, cramps, fever, helminthiasis, and infectious diseases (Ali et al. 2008). India, also called 'the spice bowl of the world', is the largest producer of ginger. Ginger has rich cultivar diversity in India, which is generally named after localities, where they are grown. More than 50 cultivars possessing varying yield and quality are grown in India with its state Kerala having more diversity followed by northeastern states of India (Ravindran et al. 2005). Major cultivars grown in India are Himachal, Maran, Assam, China, Nadia, and the exotic cultivar Rio de Janerio. Several improved varieties of ginger have also been developed which are disease resistant and high yielding. The prominent varieties developed include Athira, Rajatha, Mahima, Suprabha, Suruchi, Suravi, Himagiri, Karthika, Varada, among many others (http://iisr.agropedias.iitk.ac.in/content/varieties-ginger).

Being a herb of medicinal and culinary value, the ginger rhizomes are consumed raw. Microbiome of raw eaten vegetables and spices and the various bioactive metabolites produced by these microbes have been long ignored (Jackson et al. 2015). Endophytic fungi form a major group

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of plant microbiome, often assisting in plant growth and providing resistance against phytopathogens by the production of biologically active metabolites. These fungi live in the internal tissue of a healthy plant without apparently causing any harm to the plant. Endophytic fungi residing in ginger rhizomes and the metabolites produced by these fungi may have a possible role in the bioactivity of the ginger extracts, quality of the spice, as well can have effect on human health, as these are not removed by routine washing. These metabolites are often produced against host specific phytopathogens and may show a broad range of bioactivity which includes antibacterial, antifungal, cytotoxic, anticancerous, antioxidant, and antiinflammatory activity. Many metabolites are also found to possess plant growth promoting properties. Thus, these endophytes may also play a key role in the plant yield and disease resistance shown by improved varieties. In our previous studies, we had isolated 15 endophytic fungi from Athira (Anisha and Radhakrishnan 2015) and Mahima (unpublished data) varieties of ginger. Two isolates identified as *Acremonium* sp. and *Paraconiothyrium* sp. showed remarkable activity against ginger soft rot pathogen *Pythium myriotylum* and various clinical pathogens tested due to the production of gliotoxin and danthron, respectively. Both the metabolites identified showed a broad range of bioactivity. Thus, there is a need to study and understand the nature of endophytic fungi residing in the rhizomes of ginger in the context of both agriculture and human consumption of plant material. In the current study, we have identified the remaining endophytes and have tried to identify the bioactive metabolites produced by the isolates by metabolite analysis of fungal culture extracts using GC–MS.

Materials and methods

Isolation and identification of endophytic fungi from different varieties of ginger

Endophytic fungi were isolated from ginger variety Rio de Janerio and a Vellayanikkara variety, collected from Kerala Agricultural University, Thrissur, Kerala. The surface sterilization of rhizomes was done according to Schulz et al. (1993). Surface sterilized rhizomes were cut into small segments and incubated on Potato Dextrose Agar (PDA) media amended with 250 µg/ml streptomycin sulphate and was observed for any growth of fungi. Meanwhile, the last wash of surface sterilization was spread plated on PDA media and kept as control to check the sterility of surface sterilization procedure. For any growth on the surface sterility check plates, the experiment was repeated. The isolated fungi were further identified morphologically and by molecular methods. Previously isolated endophytic fungi from ginger varieties Athira and Mahima, collected from Kerala Agricultural University, were also identified and studied in the current work.

The endophytic fungal isolates were morphologically studied by slide culture technique. The slides stained with trypan blue were observed under 100× oil immersion. Images were processed using the Q imaging software. Molecular identification of the isolates was done by sequencing internal transcribed spacer (ITS) regions of the fungal genome. The genomic DNA was isolated using both Chromous Biotech gDNA minispin kit and by CTAB method (Voigt et al. 1999). ITS region of the fungal genome was Polymerase chain reaction (PCR) amplified using primers ITS1 (5′-TCC gTA ggT gAA CCT gCg g-3′) and ITS4 (5′-TCC TCC gCT TAT TgA TAT gC-3′) (White et al. 1990). PCR was done on Sure cycler 8800 (Agilent technologies) with the initial denaturation temperature of 95 °C for 5 min, 35 cycles of denaturation at 94 °C for 1 min, annealing at 55.5 °C for 30 s, extension for 2 min at 72 °C, and with a final extension of 72 °C for 10 min. Agarose gel electrophoresis was done to confirm the PCR products formed, which were then used as template for sequencing PCR using Big Dye Terminator Sequence Reaction Ready Mix (Applied Biosystems). ITS sequences obtained were analysed using Basic Local Alignment Search Tool (BLAST), to identify the most similar sequences from Genbank.

Screening for activity against *Pythium myriotylum*

Dual culture technique was done to screen the endophytic fungi for activity against *P. myriotylum*. Endophytic isolates were grown on PDA plates near to its edge for 7 days at room temperature. After growth of the endophyte, the phytopathogen, *P. myriotylum* was inoculated near to the opposite edge of the plate and was observed for its growth for 3–5 days. PDA plate with *Pythium* alone was kept as control. Percentage inhibition of fungal growth was calculated using the formulae: $\mathrm{PI} = (R_1 - R_2)/R_1 \times 100$, where R_1 and R_2 are the radial diameter of the phytopathogenic fungal colony in the control plate and dual culture plate, respectively (Rabha et al. 2014).

Screening for activity against clinical pathogens

To screen for activity against clinical pathogens, the endophytes were first grown in 250 ml of Potato Dextrose Broth (PDB), by inoculating an agar block (0.5 × 0.5 cm) of actively growing fungi into PDB. The fungal cultures were then incubated at room temperature for 21 days following which it was filtered through four layers of cheese cloth and then with filter paper to obtain mycelia free filtrate. The clear filtrates were then extracted thrice with

equal volume of ethyl acetate. Ethyl acetate extract was concentrated at 45 °C on a vacuum rotary evaporator. The final extract obtained was dissolved in 1 ml methanol. The antibacterial activity of these extracts was checked against *Bacillus subtilis* (MTCC 121), *Escherichia coli* (MTCC 723), *Klebsiella pneumoniae* (MTCC 109), *Staphylococcus aureus* (MTCC 96), and *Salmonella enterica typhimurium* (MTCC 1251).

Metabolite analysis of endophytic extracts for bioactive metabolites

To identify bioactive metabolites produced by the endophytic fungi, the culture extracts were subjected to Gas Chromatography–Mass Spectrometry (GC–MS) analysis on an Agilent technologies-7890 GC system. To the GC system provided with 30 m × 0.25 mm inner diameter, 0.25-µm film thickness Agilent 190913-433 column, 1 µl of the sample was injected. GC oven was held at 100 °C and then ramped from 100 to 250 °C at 5 °C/min. The chromatogram and the mass spectra were recorded and analysed by comparing with the NIST library. Compounds identified were then literature searched for their biological activity.

Results

Isolation and identification of endophytic fungi from different varieties of ginger

Three endophytic fungal isolates (GFA1, GFA2, and GFA3) were obtained from the ginger variety Rio de Janerio and one isolate (GFV1) was obtained from Vellayanikkara variety. On molecular identification, the ITS sequence of GFA1 showed maximum similarity of 84% to *Trichothecium* sp., GFA2 was 82% identical to an uncultured fungus clone and GFA3 was 100% similar to *Colletotrichum crassipes*. Previously isolated endophytes from the variety Athira (GF1, GF2, GF4, GF6, and GF8) and Mahima (GFM2, GFM3, GFM5, GFM8, GFM10, GFM11, and GFM12) were also identified. Sequences of isolates GF1 and GF2 and also its morphology were found to be similar and were found to show 99% similarity to Ascomycota sp. on BLAST analysis. Its morphology was identified as that of *Acremonium* sp. Isolate GF4 showed 99% similarity to *Fusarium* sp., whereas GF6 was 99% similar to *Gliocladiopsis* sp. and GF8 was 99% similar to *Acremonium furcatum*. Isolate GFM2 from Mahima showed 100% similarity to *Aspergillus niger*, and GFM3 was 99% similar to an uncultured fungal clone and also to *Phlebia* sp. Isolates GFM5 and GFM8 showed 100% similarity to *Fusarium oxysporum* and 99% similarity to *Earliella scabrosa,* respectively. ITS sequence of GFM10 was 82% similar to an uncultured fungal clone, while GFM11 was 99% similar to *Pseudolagarobasidium* sp. and GFM12 was 99% similar to an uncultured fungus clone. GFM3, GFM8, GFM11, and GFM12 were found to come under the phylum Basidiomycota. The ITS sequences obtained were submitted to the GenBank (Table 1). The fungal morphology and slide culture were also studied to draw-in conclusions regarding the identity of fungal isolates (Figs. 1, 2).

Screening for bioactivity of endophytic fungi

Seven isolates were found to show activity against *P. myriotylum* on dual culture. GF6 showed maximum inhibition of 71% followed by GFM3 (58%), GFM12 (56%), GFV1 (53.3%), GFM2 (51.2%), GFM5 (44%), and GF4 (36.6%). Antibacterial activity was shown only by two isolates, GFM5 and GFV1. The bioactivity exhibited by the endophytic isolates is summarized in Table 2.

Metabolite analysis of endophytic extracts for bioactive compounds

The GC–MS analysis of the fungal crude extracts of seven isolates revealed presence of bioactive volatile compounds (Table 3; Fig. 3). The isolates GFM12 and GFV1 were found to produce tyrosol. GFV1 also produced benzene acetic acid and dehydromevalonic lactone. Other compounds produced were ergone by GF6 and *N*-aminopyrrolidine by GFM12. Among the isolates, six were found to produce bioactive fatty acids and its derivatives. Isolates GFA1, GFA2, GFM10, GFM11, GFM12, and GFV1 produced *n*-hexadecanoic acid. GFV1 produced myristic acid and methyl linoleate. Isolates GFM10 and GFV1 were found to produce palmitic acid methyl ester. GFM11, GFM12, and GFV1 produced linoleic acid, where as oleic acid production was exhibited by GFM12 and GFV1.

Discussion

Fifteen endophytic fungal isolates belonging to 14 different genera were identified in the study. Four isolates were identified from the variety Athira. An isolate previously identified in our study from Athira makes a total of five endophytic fungi from the variety. Seven isolates identified in the current study from the variety Mahima and one previously identified make a total of eight isolates from Mahima. Rio de Janerio and Vellayanikkara variety yielded three and one endophytes, respectively. Thus, the variety Mahima was found to harbor more diverse fungi followed by Athira. The morphological and molecular identification

Table 1 Endophytic fungi from *Z. officinale* with their closest match as identified by BLAST analysis and the accession numbers of the partial ITS sequences of the endophytic isolates submitted to GenBank

Endophytic fungi	Closest NCBI match	Percentage identity	Submitted to NCBI with accession no.
GF1/GF2	*Ascomycota* sp.	99	KX247110
GF4	*Fusarium* sp.	99	KX247111
GF6	*Gliocladiopsis*	99	KX247112
GF8	*Acremonium furcatum*	99	KX247113
GFA1	*Trichothecium* sp.	84	KX247122
GFA2	Uncultured fungus clone	82	KX247123
GFA3	*Colletotrichum crassipes*	100	KX247124
GFM2	*Aspergillus niger*	100	KX247114
GFM3	Uncultured fungus clone/*Phlebia* sp.	99	KX247115
GFM5	*Fusarium oxysporum*	100	KX247116
GFM8	*Earliella scabrosa*/uncultured fungus	99	KX247117
GFM10	Uncultured fungus clone	82	KX247118
GFM11	*Pseudolagarobasidium* sp.	99	KX247119
GFM12	Uncultured fungus clone/*Cerrena* sp.	99	KX247120
GFV1	Fungal sp.	97	KX247125

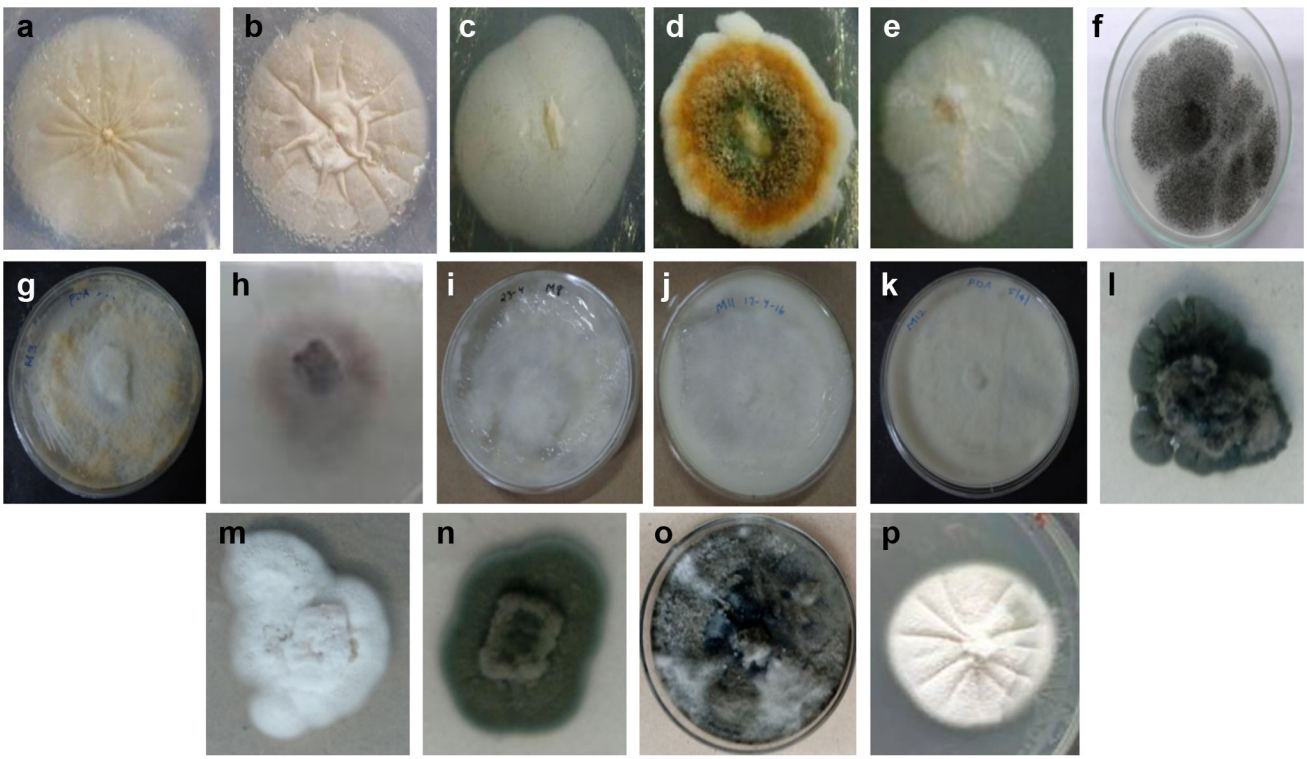

Fig. 1 Endophytic fungi from *Z. officinale* cultured on PDA plates: **a** GF1, **b** GF2, **c** GF4, **d** GF6, **e** GF8, **f** GFM2, **g** GFM3, **h** GFM5, **i** GFM8, **j** GFM11, **k** GFM12, **l** GFM10, **m** GFA1, **n** GFA2, **o** GFA3, and **p** GFV1

studies were coinciding. The different genera of fungi identified were *Acremonium* sp., *Gliocladiopsis* sp., *Fusarium* sp., *Colletotrichum* sp., *Aspergillus* sp., *Phlebia* sp., *Earliella* sp., and *Psuedolagarobasidium* sp. For some fungi, identification to the genus level was not possible. Though Ginting et al. (2013) had isolated and identified many endophytic fungi from different parts of red ginger from Indonesia, comprehensive work on the identification

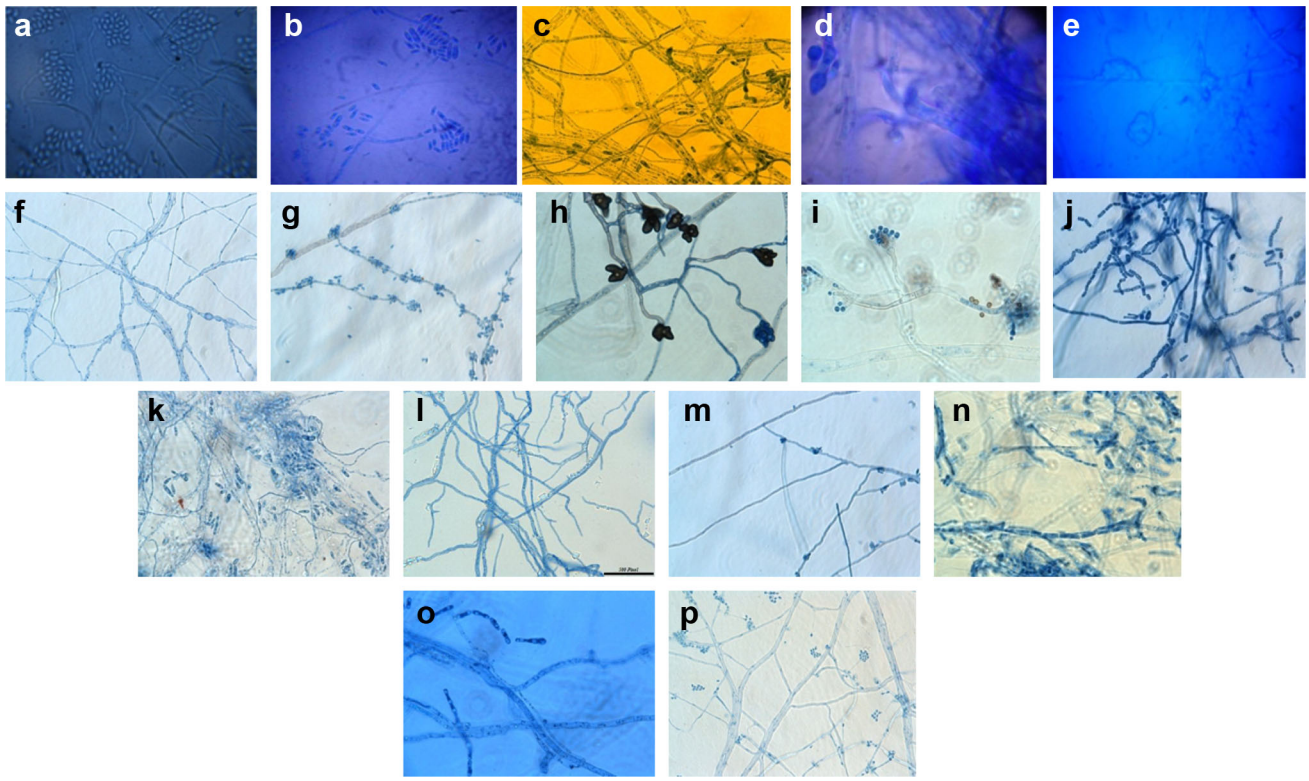

Fig. 2 Microscopic images of endophytic fungal slide cultures: **a** GF1, **b** GF2, **c** GF4, **d** GF6, **e** GF8, **f** GFA1, **g** GFA2, **h** GFA3, **i** GFM2, **j** GFM3, **k** GFM5, **l** GFM8, **m** GFM10, **n** GFM11, **o** GFM12, and **p** GFV1

Table 2 Percentage inhibition of *Pythium* and antibacterial activity [zone of inhibition (mm)] shown by the endophytic fungal extracts

Endophytic fungi	*P. myriotylum* (% inhibition)	Zone of inhibition (mm)				
		S. aureus	*B. subtilis*	*S. typhimurium*	*K. pneumoniae*	*E. coli*
GF2	–	–	–	–	–	–
GF4	36.66	–	–	–	–	–
GF6	71.11	–	–	–	–	–
GF8	–	–	–	–	–	–
GFA1	–	–	–	–	–	–
GFA2	–	–	–	–	–	–
GFA3	–	–	–	–	–	–
GFM2	51.22	–	–	–	–	–
GFM3	57.97	–				
GFM5	44	14	18	–	–	–
GFM8	–	–	–	–	–	–
GFM10	–	–	–	–	–	–
GFM11	–	–	–	–	–	–
GFM12	56	–	–	–	–	–
GFV1	53.33	20	10	10	–	–

of endophytic fungi from ginger grown in the Indian subcontinent has not been reported so far. In our previous work, we had identified a very potent *Acremonium* sp. from the ginger variety Athira which showed promising bioactivity. Thus, this genus may be concluded as the most frequently isolated fungi from the variety Athira.

Table 3 Bioactive compounds in the endophytic fungal extracts as identified by GC–MS

Compound	Endophytic fungi and the relative abundance of the compound
Dehydromevalonic lactone	GFV1 (3.64%)
Benzene acetic acid	GFV1 (1.64%)
Benzeneethanol, 4-hydroxy-(tyrosol)	GFV1 (3.39%), GFM12 (0.16%)
Tetradecanoic acid (myristic acid)	GFV1 (0.93%)
Hexadecanoic acid	GFV1 (1.16%), GFM10 (1.36%)
Methyl ester (palmitic acid methyl ester)	GFV1 (1.75%), GFM10 (3.10%)
n-Hexadecanoic acid	GFV1 (18.65%), GFM12 (13.44%), GFM11 (26.06%), GFA1 (8.54%), GFA2 (26.11%), GFM10 (18.45%)
9,12-Octadecadienoic acid (*Z*,*Z*)-, methyl ester (methyl linoleate)	GFV1 (0.89%)
9,12-Octadecadienoic acid (*Z*,*Z*) (linoleic acid)	GFV1 (6.12%), M12 (6.79%), M11 (26.45%)
Oleic acid	GFV1 (13.24%), M12 (60.68%)
N-Aminopyrrolidine	GFM12 (0.14%)
Ergosta-4, 6, 8(14), 22-tetraen-3-one (ergone)	GF6 (84.53%)

Acremonium sp. are reported as major grass endophytes (White 1987) and also have wide occurence as endophytes in many non-grass plants such as *Rhizophora apiculata* (Rukachaisirikul et al. 2012), *Vitis vinifera* (Arnone et al. 2009), *Actinidia macrosperma* (Lu et al. 2012), *Bursera simaruba* (González et al. 2016), and many more. *Gliocladiopsis* sp. has been reported as endophyte from *Paris polyphylla* (Li et al. 2008) and *Rafflesia cantleyi* (Refaei et al. 2011). *Fusarium* sp. has a wide occurrence as endophyte in many plant species including *Drepanocarpus lunatus* (Liu et al. 2016), *Axonopus compressus* (Zakaria and Ning 2013), *Nothapodytes foetida* (Musavi and Balakrishnan 2013), *Dioscorea zingiberensis* (Li et al. 2014), *Solanum lycopersicum* (Aimé et al. 2013), *Juniperus recurva*, *Catharanthus roseus* (Kumar et al. 2013), *Ginkgo biloba* (Cui et al. 2012), etc. *Colletotrichum* sp. is another very frequently isolated endophyte and the isolate *C. crassipes* identified in the study has been reported from *Artemisia* sp. (Huang et al. 2009), *Phyllanthus amarus* (Kandasamy et al. 2015), and *Tinospora cordifolia* (Mishra et al. 2012). *Aspergillus niger* is reported as endophyte from *Tabebuia argentea* (Channabasava and Govindappa 2014), *Heteroscyphus tener* (Li et al. 2013), *Avicennia marina* (Liu et al. 2013), *Opuntia dillenii* (Ratnaweera et al. 2015), *Entandrophragma congoense* (Sandhu et al. 2014), and many more. The remaining genera identified including *Phlebia* sp., *Earliella* sp., and *Pseudolagarobasidium* sp. comes under the phylum Basidiomycota. Basidiomycetes, though reported as endophytes, are less studied (Martin et al. 2015) and are not very commonly isolated like Ascomycetes. This is the first report of members of Basidiomycota from ginger rhizomes. *Phlebia* sp. has been reported as endophyte from *Piper hispidum* (Orlandelli et al. 2012), *Hevea* (Martin et al. 2015), and *Elaeis guineensis* (Anuar et al. 2015). While *Earliella scabrosa* has been isolated from *Pinus densiflora* (Kil et al. 2009). *Pseudolagarobasidium* sp. as an endophyte is reported from *Bruguiera gymnorrhiza* (Wibowo et al. 2016).

In the bioactivity analysis of the endophytic fungi, seven isolates from the 15 identified (nearly 50%) showed activity against *P. myriotylum,* but only two isolates showed antibacterial activity against clinical pathogens. Isolate GF6, identified as *Gliocladiopsis* sp., showed a significant inhibition of *P. myriotylum* followed by *Phlebia* sp. GFM3, uncultured fungus clone GFM12, Fungal sp. GFV1, *Aspergillus* sp. GFM2, *Fusarium* sp. GFM5, and *Fusarium* sp. GF4. Fungal sp. GFV1 and *Fusarium* sp. GFM5 were the isolates which showed activity against bacterial isolates. Crude extract of *Gliocladiopsis* sp. isolated from *R. cantleyi* is previously reported to inhibit the growth of *Candida albicans* (Refaei et al. 2011). Extracts of endophytic *A. niger* have also been reported to have activity against *C. albicans* (Ratnaweera et al. 2015). *Aspergillus niger* from *Entandrophragma congoense* produced a dimeric naphtho-γ-pyrone, 2-hydroxydihydronigerone along with nigerone, pyrophen, kojic acid, 4-(hydroxymethyl)-5-hydroxy-2H-pyran-2-one, and *p*-hydroxyphenylacetic acid. All the compounds exhibited weak antibacterial activity against gram-negative bacteria (Happi et al. 2015). Endophytic *Aspergillus niger* has also been reported as a good source of anticancer agents such as lapachol (Channabasava and Govindappa 2014), Malformin A1 (Li et al. 2013), lovastatin, and nigerasterols A

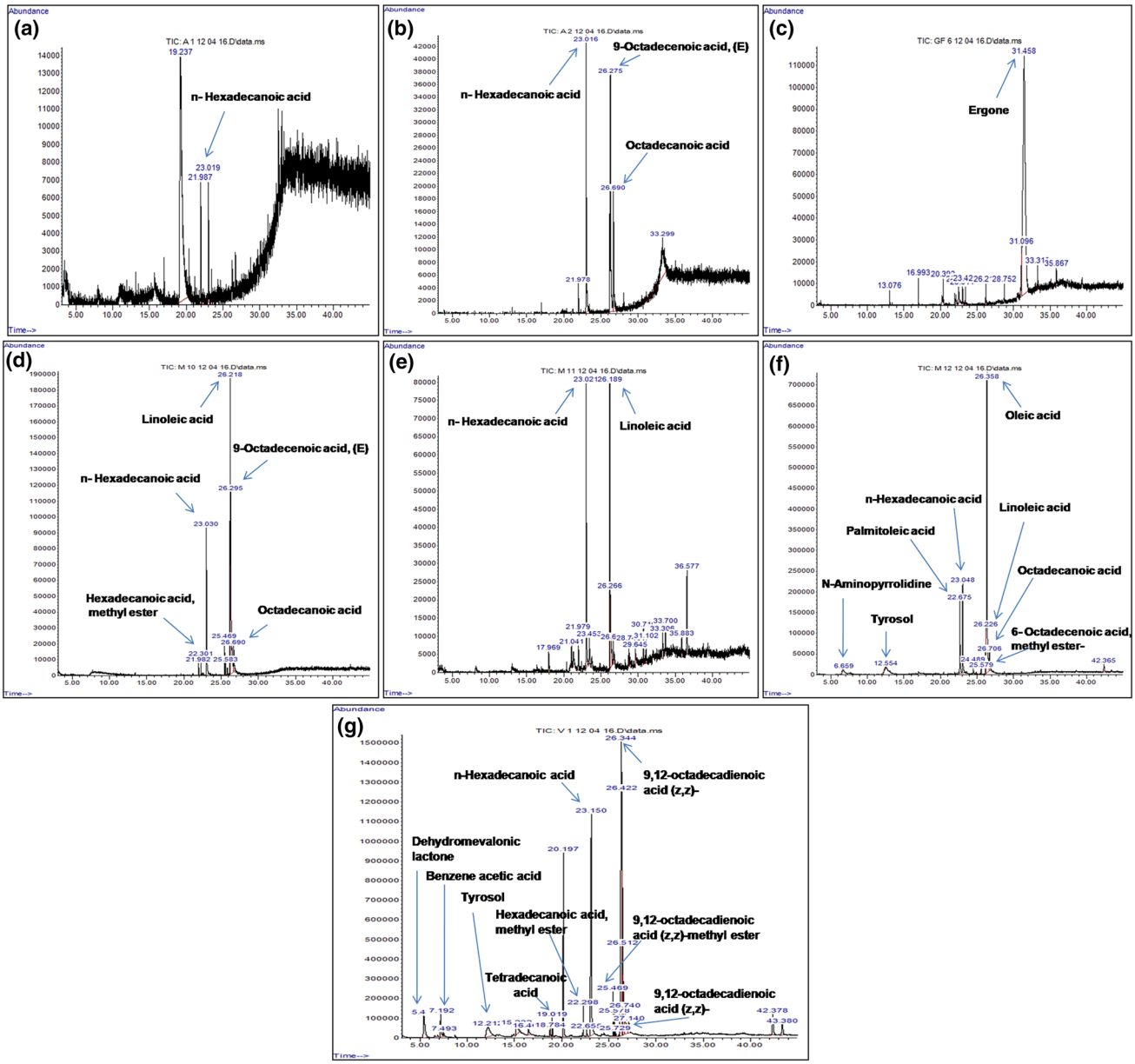

Fig. 3 Total ion chromatogram of endophytic fungal crude extracts showing the presence of bioactive compounds at various retention times: **a** GFA1, **b** GFA2, **c** GF6, **d** GFM10, **e** GFM11, **f** GFM12, and **g** GFV1

and B (Liu et al. 2013). Crude extract of endophytic *F. oxysporum* has been reported to have activity against *C. albicans* and various bacterial isolates tested (Musavi and Balakrishnan 2013). Endophytic *F. oxysporum* is also reported to produce its corresponding host metabolites such as podophyllotoxin, taxol (Elavarasi et al. 2012), ginkgolide B (Cui et al. 2012), vincristine, and vinblastin (Kumar et al. 2013), which thus indicates its close association with different hosts.

In the GC–MS analysis of endophytic crude extracts, tyrosol production was observed in the Fungal sp. GFV1 and GFM12. Tyrosol has previously been reported in endophytic culture extracts of *Diaporthe* (Specian et al. 2012), *Colletotrichum* (Bungihan et al. 2013), *Glomerella* (Guimarães et al. 2009), *Phyllosticta* (Tan et al. 2015), and *Phialocephala fortinii* (Cui et al. 2016). It has a wide range of bioactivity including plant growth promotion (Kimura and Tamura 1973), cytotoxicity (Guimarães et al. 2009), antibacterial, and antifungal (Specian et al. 2012; Brilhante et al. 2016), and is a very potent antioxidant. Interestingly, it is also the quorum sensing molecule involved in the morphogenesis of the dimorphic fungi *C. albicans* (Wongsuk et al. 2016). *N*-Aminopyrrolidine was also detected in the crude extract of GFM12. Benzene acetic

acid and dehydromevalonic lactone were two other compounds detected in Fungal sp. GFV1. Benzene acetic acid has been reported from the endophytic fungus *Chaetomium fusiforme* (Guo et al. 2008). It is a type of auxin (Wightman and Lighty 1982) and is an antimicrobial produced by metapleural glands of most ant species for controlling fungal pathogens (Fernandez-Marin et al. 2015). Dehydromevalonic lactone has been reported to be produced during the solid-state fermentation of *Monascus ruber*, and is reported to impart fragrance (Vidyalakshmi et al. 2009). The isolate *Gliocladiopsis* sp. GF6, which was most bioactive against *Pythium*, was found to produce Ergosta-4, 6, 8(14), 22-tetraen-3-one also called ergone. Ergone is a cytotoxic metabolite reported from mushrooms including *Polyporus umbellatus* (Lee et al. 2005) and *Ganoderma atrum* (Shen et al. 2008). It is also reported to show anti-aldosteronic diuretic effect (Yuan et al. 2004). Other major group of biological compound detected in many of the endophytic crude extracts was fatty acids and their derivatives. Fatty acids and their derivatives are major metabolites of many fungi including Basidiomycetes (Sumner 1973). They have been reported to possess antibacterial (Kabara et al. 1972), antifungal (Walters et al. 2004), antimalarial (Carballeira 2008), and antinematicidal activities (Stadler et al. 1994). The underlying mechanism proposed for the bioactivity of fatty acids is their ability to disrupt cell membrane (Bergsson et al. 2001). This may in turn increase the intake of other antimicrobials into the cytoplasm of pathogens.

In the current study, the highest activity against *Pythium* was exhibited by the ergone producing *Gliocladiopsis* sp. GF6 isolated from the variety, Athira. *Acremonium* sp., isolated in our previous study, displayed a strong antagonism to *Pythium* by the production of gliotoxin and was an endophyte isolated from the variety Athira. In addition, we had previously isolated a very potent endophytic bacterium from this variety which showed high activity against *Pythium* due to the production of phenazine (Jasim et al. 2014). Ginger variety Athira, developed from the cultivar Maran, is specifically tolerant to soft rot caused by *Pythium* sp. and to bacterial wilt than its parent cultivar and other varieties. The one possible reason for this may be attributed to the presence of these bioactive endophytes in the ginger rhizome. More than 60% of the isolates from variety Mahima, including previously identified *Paraconiothyrium* sp., showed activity against *Pythium*. Mahima is a high yielding variety of ginger resistant to nematodes *Meloidogyne incognita* and *M. javanica*. It is interesting to note that basidiomycetes formed a major group of endophytes isolated from this variety, and two of them *Phlebia* sp. and GFM12 showed activity against *Pythium*. GFM12, *Pseudolagarobasidium* sp., and Fungal sp. GFM10 from Mahima and Fungal sp. GFV1 from Vellayanikkara variety were found to produce linoliec acid which has been reported to have excellent nematicidal activity (Stadler et al. 1994). Linoliec acid is the most abundantly produced fatty acid by Basidiomycetes (Sumner 1973). Endophytic production of fatty acids probably can play a role in resistance of the variety Mahima against nematodes. The population of endophytic fungi in all varieties studied varied, which may be due to difference in the genotype of the plant or environmental conditions. The endophytes may thus be a contributing factor in the differences in the performance of various varieties of ginger.

Thus, the myriad of endophytic fungi living in ginger rhizomes and the metabolites produced by them can impart a synergistic effect on the pathogens of ginger. An understanding of these metabolites is essential in the context of consumption of ginger as a medicine and spice.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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