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ORIGINAL ARTICLE

Salt tolerance of endophytic *Trichoderma koningiopsis* YIM PH30002 and its volatile organic compounds (VOCs) allelopathic activity against phytopathogens associated with *Panax notoginseng*

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Abstract *Trichoderma* spp. are widely used in the biological control of plant pathogens due to their high antagonistic and mycoparasitic potential. However, interaction among phytopathogens–*Trichoderma*–plant–soil is very complex and needs further investigation. In this study, we report the high salt-tolerant and mycoparasitic properties of endophytic *T. koningiopsis* YIM PH30002, isolated from the root of 2-year-old healthy Sanqi (*Panax notoginseng*), and its allelopathic antagonistic activity against phytopathogens associated with the host plant. *Trichoderma koningiopsis* YIM PH30002 exhibited significant inhibition of the growth of four host root-rot phytopathogens, *Phoma herbarum*, *Fusarium flocciferum*, *Scytalidium lignicola*, and *Epicoccum nigrum*, by covering the colony of phytopathogens, coiling and twisting the mycelium in a probable mechanism of mycoparasitism, producing volatile organic compounds (VOCs). In potato dextrose broth (PDB) culture medium, *T. koningiopsis* YIM PH30002 produced at least ten kinds of volatile substances which were identified as alkanes, monoterpenes and arenes, heterocycles, and aldehydes by GC-MS. The results indicate that YIM PH30002 can exert antagonistic actions by integrated ways to help its host defend diseases, and could be used as a promising candidate for the biological control of *P. notoginseng* root-rot disease.

Keywords *Trichoderma koningiopsis* · Salt tolerance · Allelopathic antagonistic · Mycoparasitism · *Panax notoginseng*

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Introduction

The biological control of plant diseases (Vitale et al. 2012; Peng et al. 2014; Troian et al. 2014; Zhang et al. 2014) is attracting increasing interest from researchers as chemical pesticides showed high costs and low control efficiencies, especially for soil-borne diseases (Harrier and Watson 2004). Meanwhile, agrochemicals result in other serious problems, such as soil and water pollution, food safety, tolerant or antagonistic harmful microbes, etc. *Trichoderma* spp. are ubiquitous in soil environments, and widely developed as biopesticides, biofertilizers, and soil amendments due to their abilities to protect plants, enhance plant growth, and control pathogen population (Vinale et al. 2008; Vujanovic et al. 2012; Zhang et al. 2014). To date, hundreds of different *Trichoderma*-based preparations have been commercially used to protect or increase the productivity of various crops (Delabona et al. 2012; Rinu et al. 2014).

Panax notoginseng (Burkill) F.H. Chen (Sanchi), an important traditional Chinese medicinal plant, is mainly cultivated in Wenshan, Yunnan Province. Saponins of *P. notoginseng* (PNS) are considered as the major active ingredients of notoginseng, and extensively used as a therapeutic agent in China, responsible for a wide range of pharmacological efficacies, including

hemeostatic, antioxidant, neuroprotective, antitumor, antidiabetic, etc. (Wang et al. 2014; Yang et al. 2014; Zeng et al. 2014). Because of its low adaptive capacity to the environment, *P. notoginseng* is strictly demanding on climate and environment, thus mainly distributing in middle and high elevation areas around the subtropical zone (You et al. 2010). Soil conditions are required to be porous, rich in organic matter, and moist. However, long growth period (2–4 years), shade, and humid planting conditions facilitate the infection of this plant by numerous phytopathogens. Among the diseases of *P. notoginseng*, the root-rot disease complex (RRDC) is the most destructive, as it results in yield reduction and low content of active ingredients (Miao et al. 2006). RRDC of *P. notoginseng* was reported to be caused by fungal pathogens, including *Alternaria panax*, *A. tenuis*, *Cylindrocarpon destructans*, *C. didynum*, *Fusarium solani*, *F. oxysporum*, *Phoma herbarum*, and the chromist *Phytophthora cactorum* (Miao et al. 2006; Miao et al. 2015). To achieve higher yield and control diseases, vast and successive agrochemical fertilizers and fungicides are widely applied in plantation. Consequently, these approaches have brought about many negative environmental problems, such as soil salinization (Moebius-Clune et al. 2011; Singh et al. 2011), which can, conversely, suppress the crop growth seriously. Soil microbial biodiversity and the structures and functions of microbial communities were also affected severely, resulting in fungicide-tolerant pathogens and significant continuous cropping obstacles (Liu et al. 2011). Thus, integration with biological strategies can be a sustainable way to achieve reliable control of complex diseases in *P. notoginseng*.

Endophytic fungi colonizing living plants without causing negative effects are likely to produce natural metabolites, which endow selective merits to the host in the aspects of enabling more medicinal value to the host pharmaceutical plant (Cao et al. 2015), suppressing the development of other microorganisms (Zhang et al. 2006) or by making the host unattractive to predators (Strobel and Daisy 2003). In our program of seeking effective potential biocontrol agents for *P. notoginseng*, endophytic fungi *T. koningiopsis* YIM PH30002, isolated from healthy root of notoginseng plants, showed mycoparasitic activity to the notoginseng root-rot pathogens *Phoma herbarum*, *Fusarium flocciferum*, *Scytalidium lignicola*, and *Epicoccum nigrum*. In this study, we try to determine its volatile organic compounds (VOCs) allelopathic antagonism to notoginseng root-rot pathogens and salt-tolerant properties in the resistance of abiotic stress. Furthermore, the pathogenicity of YIM PH30002 to *P. notoginseng* was also tested in this report, as certain fungal endophytes could cause disease for host plants (Malcolm et al. 2013) in some cases, which indicate the necessity to reverify the possible pathogenicity of potential biocontrol microorganisms to target plants.

Materials and methods

Fungal isolation and identification

The endophytic fungus *T. koningiopsis* YIM PH30002 used in this study was isolated from a 2-year-old healthy *P. notoginseng* root cultivated in Wenshan, China, in August 2012. The surface sterilization and isolation of fungal endophytes followed the procedures described by Park et al. (2012). Plant samples were washed thoroughly with running tap water to remove soil particles and rinsed six times with distilled water. The tissues were surface-disinfected in 70 % ethanol for 1 min, 5.5 % sodium hypochlorite for 1 min, and plated on potato dextrose agar (PDA) after rinsing with sterile water three times. After 5 days of incubation at 28 °C, hyphal tips grown out of the tissues were transferred to fresh PDA and incubated at 28 ± 1°C for 7 days to form pure fungal colonies. Among the colonies, isolate YIM PH30002 was selected and used for this study as it showed mycoparasitic potential to some phytopathogens.

The identification of isolate YIM PH30002 was based on the morphological characterization and molecular data. DNA was extracted from 0.5 to 1.0 g chilled mycelium in liquid nitrogen using the SDS-CTAB method (Kim et al. 1990). An internal transcribed spacer (ITS) region was amplified using primers ITS 1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS 4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al. 1990). The PCR reaction was performed in a 50-μL reaction mixture containing 1 μL of template DNA, 1 μL forward primer (10 μM), 1 μL reverse primer (10 μM), 5 μL reaction buffer (10×), 4 μL dNTPs (2.5 μM), 0.5 μL of *Taq* DNA Polymerase (500 U), and 37.5 μL sterile double-distilled water. The PCR cycling protocol consisted of an initial denaturation at 94 °C for 4 min, followed by 30 cycles of 94 °C for 1 min, 56 °C for 1 min, and 72 °C for 2 min, and a final elongation step of 72 °C for 10 min. As a negative control, the template DNA was replaced by sterile double-distilled water. The PCR-amplified products were separated by agarose gel electrophoresis, and sequenced on an ABI PRISM 3730 sequencer at Sangon Biotech (Shanghai, China). The BLAST-sequenced data have been deposited at GenBank (accession no. KM190127) and a voucher specimen was preserved at the Yunnan Institute of Microbiology, Kunming, China.

Phytopathogens in this study were also isolated from the rotten-root of *P. notoginseng* and identified as *Phoma herbarum* YIM PH30340, *F. flocciferum* YIM PH30355, *S. lignicola* YIM PH30094, and *E. nigrum* YIM PH30306 based on morphological and molecular characteristics. Their pathogenicity to *P. notoginseng* was confirmed by Miao et al. (2015). All the fungi were maintained or cultured on PDA medium and in liquid potato dextrose broth (PDB) medium.

Pathogenicity of *T. koningiopsis* YIM PH30002

The pathogenic abilities of *T. koningiopsis* YIM PH30002 was estimated with 1-year-old healthy *P. notoginseng* as the host plants in greenhouse tests. Six replications for each treatment with three *P. notoginseng* plants were carried out in pot trials. Ten-liter (0.6 m × 0.5 m × 0.125 m, L/W/H) containers were used in the greenhouse treatments. Spores of the *T. koningiopsis* YIM PH30002 were prepared on PDA medium. A 6-L volume of sterilized soil mixture (6 L of soil and 100 mL of dH_2O) mixed with 2.0×10^{12} spores of *T. koningiopsis* YIM PH30002 was poured into each container, covered with 100 g dry pine needle. The control was represented by the soil without inoculation with YIM PH30002. All the containers (2 treatments × 6 replicates = 12 containers) were incubated for 3 months at 25 °C. *Panax notoginseng* plants were harvested and the health of roots was visually inspected.

Resistance to salt stress of *T. koningiopsis* YIM PH30002

Salt tolerance was determined by measuring the biomass production, by cultivating *T. koningiopsis* at a range of different NaCl concentrations in PDB medium. A 500-μL *T. koningiopsis* spore suspension (about 10^6 CFU/mL) was inoculated in 250-mL conical flasks containing 75 mL sterile PDB medium amended with sodium chloride (NaCl) concentrations (w/v) of 0, 2, 4, 6, 8, 10, 13, 15, 17, 19, 21, 23, 25, 27, 29, and 30 percentages. Each salt concentration was performed in triplicate. The flasks were incubated on a rotary shaker set (XWW 25/450, Changzheng Company, Leshan, China), at 28 °C and 130 rpm for 15 days. The growth and morphological characteristics were compared and recorded daily. Mycelium was collected by filtering through glass fiber (GF/C filters, Whatman) and dried to constant weight at 100 °C.

Dual culture and mycoparasitism

The effect of strain YIM PH30002 on the growth and morphology of the phytopathogens was assayed by dual culture tests (Dennis and Webster 1971a). The plates were incubated at 28 °C and growth was observed and recorded every 12 h for 5 days. The percent growth rate was calculated by the following formula:

$$I = \frac{C\text{-}T}{C} \times 100\% \quad (1)$$

where: I = % inhibition in mycelium growth; C = growth of pathogen in control plates; T = growth of pathogen in dual culture plates.

The mycoparasitism of *T. koningiopsis* was observed by collecting the hyphae from the interaction zone and transferred on glass cover lips, fixed overnight at 4 °C in solution of 2.5 % glutaraldehyde, dehydrated in a series of ascending ethanol concentrations (50–100 %) (v/v), dried in a cool dry place, and coated with gold in a sputter coater (SCD 005, BAL-TEC, Czech Republic), and examined with a scanning electron microscope (Quanta 200 FEG, FEI Company, USA).

Antagonistic assay of VOCs from *T. koningiopsis* YIM PH30002 against pathogens

The allelopathic antagonistic activity of VOCs produced by *T. koningiopsis* YIM PH30002 was determined using the methods described by Dennis and Webster (1971b). The test phytopathogens were grown on PDA in petri dishes for 7 days as stock cultures. Agar disks (diameter 6 mm) were cut from the margin of stock cultures, put on the center of PDA medium, and incubated for 72 h at 28 °C. The lid of each dish was replaced by a bottom containing PDA inoculated with *T. koningiopsis* YIM PH30002 grown for 48 h. The dishes

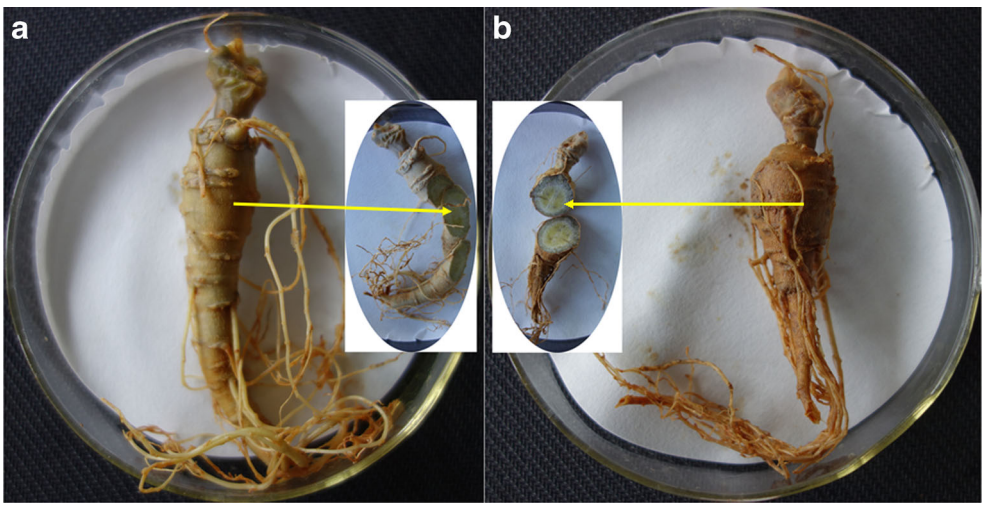

Fig. 1 Pathogenic estimation of *T. koningiopsis* YIM PH30002 on *P. notoginseng* plants for 3 months: **a** *P. notoginseng* plants inoculated with *T. koningiopsis* YIM PH30002; **b** the control

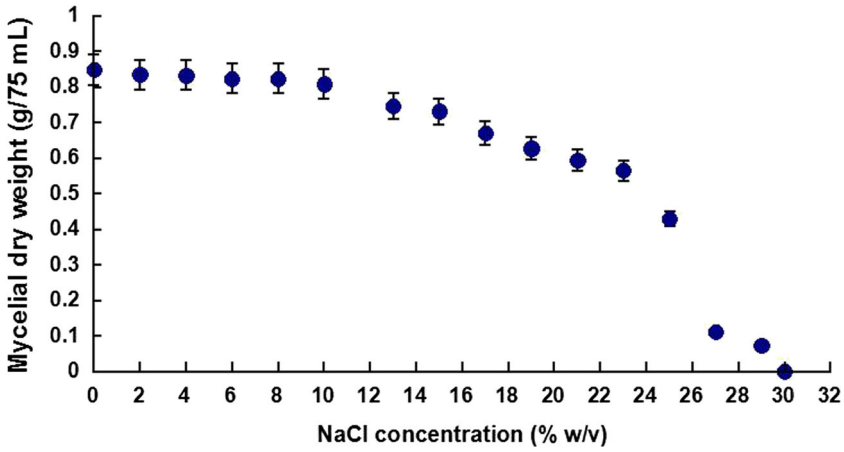

Fig. 2 Dry weight of *T. koningiopsis* mycelia from the PDB medium amended with incremental NaCl concentrations after 15 days of cultivation. The standard deviation lines represent variation of the mean value obtained from three replicate cultivations

were taped together with laboratory film. The controls without *T. koningiopsis* were also replaced in the same way. The tests were conducted in four replicates.

Growth of phytopathogens was recorded every 12 h after co-inoculation. After 5 days incubation, the colony diameter of the phytopathogenic fungi was measured and compared to that on the control according to the above formula (1).

VOCs analysis by GC-MS

Collection of the VOCs from the culture of *T. koningiopsis* was performed by HS-SPME. *Trichoderma koningiopsis* was grown in a 15-mL vial containing 5 mL PDB medium at 28 °C for 5 days. Extraction was carried out at 50 °C for 30 min with preconditioned PA fiber (85 μM, polyacrylate) in the headspace. The VOCs were desorbed by placing the fiber into the gas chromatography (GC) injection port for 5 min. The analyses were conducted by GC-MS (7890GC/5975MSD, Agilent, USA), using an HP-5ms capillary column (0.25 m × 30 m), at 50 °C for 2 min, then at a rate of 6 °C/min to 180 °C. Subsequently, a temperature of 280 °C was reached at a rate of 8 °C/min, and kept for 5 min. The helium flow was 1.0 mL/min, and mass spectrometer monitoring in full scan mode (*m*/*z* 35–550) operated in the electron ionization mode at 70 eV with a source temperature of 220 °C. Volatile compounds were identified by comparison with the National Institute of Standards and Technology (NIST) database. The VOCs that showed mass spectra with match factor ≥90 % were considered as identified substances.

Results

Pathogenicity to roots of *P. notoginseng*

Trichoderma koningiopsis YIM PH30002 showed no pathogenic activity in greenhouse estimations. After co-inoculation for 3 months, *P. notoginseng* plants remained in the healthy growing status in both containers with *T. koningiopsis* YIM PH30002 as well as the control, and the spores and mycelia were clear at the interspace of soil. The roots of *P. notoginseng* showed were shown to be symptomless on the surface and inner tissues (Fig. 1a, b) when compared with the control, which indicates that the *T. koningiopsis* YIM PH30019 was harmless to *P. notoginseng*.

Salt-tolerant properties

Trichoderma koningiopsis YIM PH30002 could grow in almost all the tested NaCl concentrations, except 30 %, but grew well from 0 to 10 % without significant differences (Fig. 2 and Table 1). The morphology and color of the fermentation broth

Table 1 Mycelial dry weight of *T. koningiopsis* cultured in PDB medium amended with different NaCl concentrations

NaCl concentration (%)	Weight[a] (g/75 mL)
0	0.849
2	0.835
4	0.833
6	0.823
8	0.823
10	0.809
13	0.746
15	0.732
17	0.671
19	0.628
21	0.595
23	0.565
25	0.429
27	0.113
29	0.073
30	0

[a] Mean value of three replications

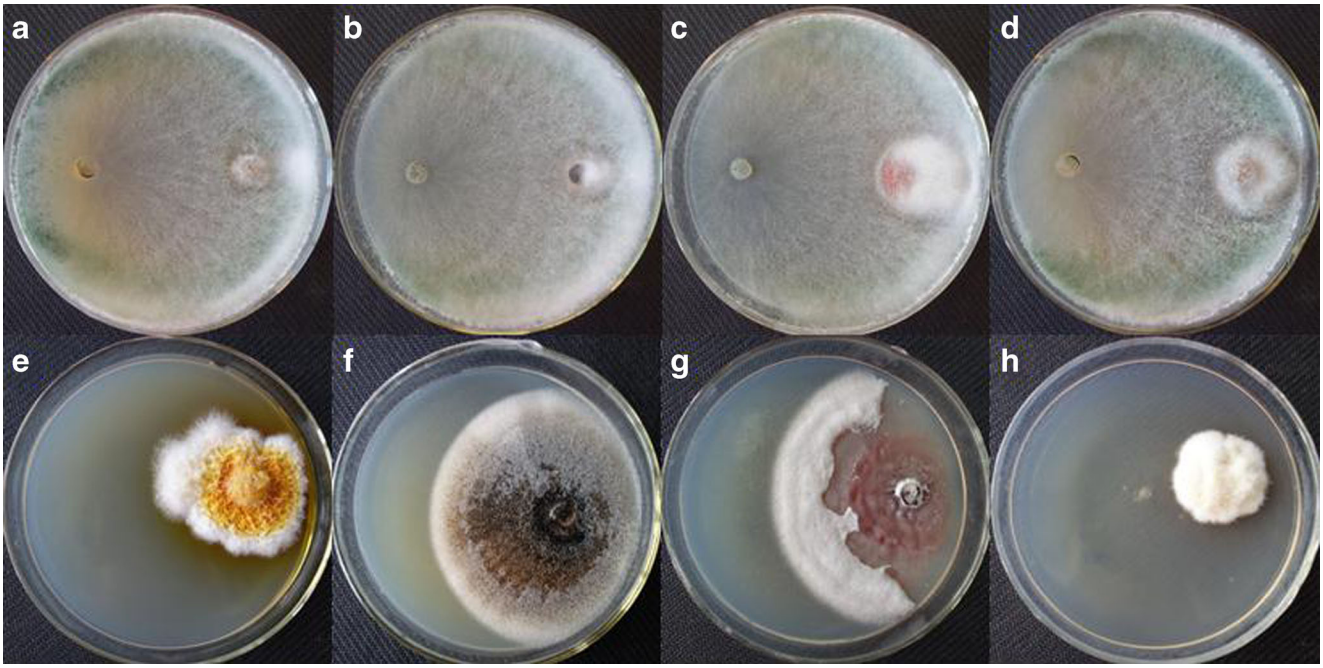

Fig. 3 Dual cultures collected 5 days after inoculation of *T. koningiopsis*. *Trichoderma koningiopsis* was cultured on the left side, while phytopathogens were cultured on the right: **a** *E. nigrum*; **b** *S. lignicola*; **c** *Phoma herbarum*; **d** *F. flocciferum*. **e**, **f**, **g**, and **h** are the corresponding controls of **a**, **b**, **c**, **d**, respectively

changed clearly with the increase of salinity. The color of the culture broth was light yellow and faded gradually, with homogeneous spherical mycelial granules at concentrations in the range 2–8 %, and was slightly dark with mushy mycelia at 10–15 %. When the saline concentration reached 27 %, mycelial growth occurred only slightly. These results indicated that *T. koningiopsis* YIM PH30002 had high osmotolerance in its living environment.

Mycoparasitism in dual culture

There were no obvious inhibition halos in the treatment plates, but the growth of four test phytopathogens were effectively confined by *T. koningiopsis* YIM PH30002 compared with the corresponding controls (pathogenic fungi without *T. koningiopsis*) at 5 days (Fig. 3). *Trichoderma koningiopsis* exhibited probable parasitic activities to the tested pathogens. Scanning electron microscopy was taken for detailed investigation of the interaction between *T. koningiopsis* YIM PH30002 and the tested pathogens (*E. nigrum* and *F. flocciferum*) (Figs. 3 and 4). The results brought further insights into the events leading to pathogen growth inhibition. Examination of hyphae interaction (Fig. 4) sampled from 5-day-old dual cultures showed that the hyphae of *T. koningiopsis* could grow alongside and coiling around the pathogens' hyphae. In the dual culture of *Phoma herbarum*, the *Trichoderma* hyphal tips grew into thin branches

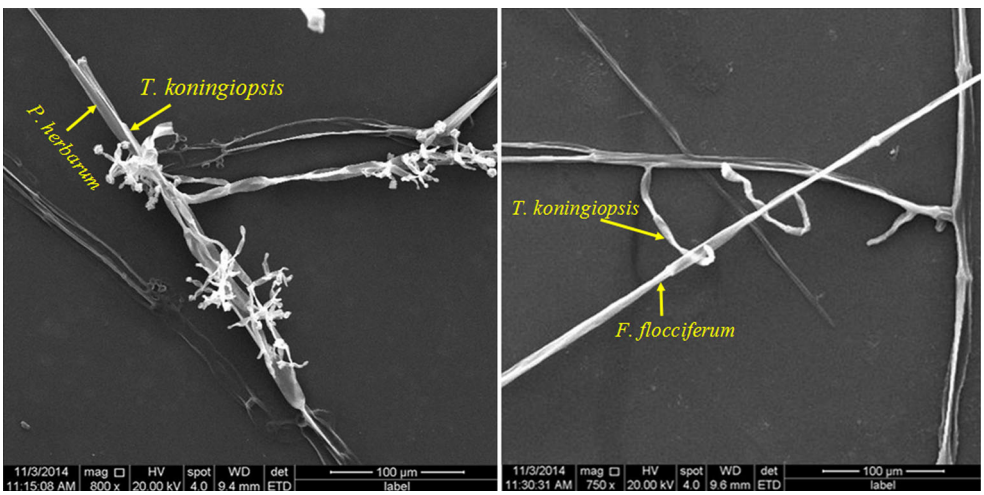

Fig. 4 Scanning electron micrographs of mycelial samples of two *T. koningiopsis*-parasited phytopathogens at 5 days: **a** *T. koningiopsis* and *Phoma herbarum*; **b** *T. koningiopsis* and *F. flocciferum*

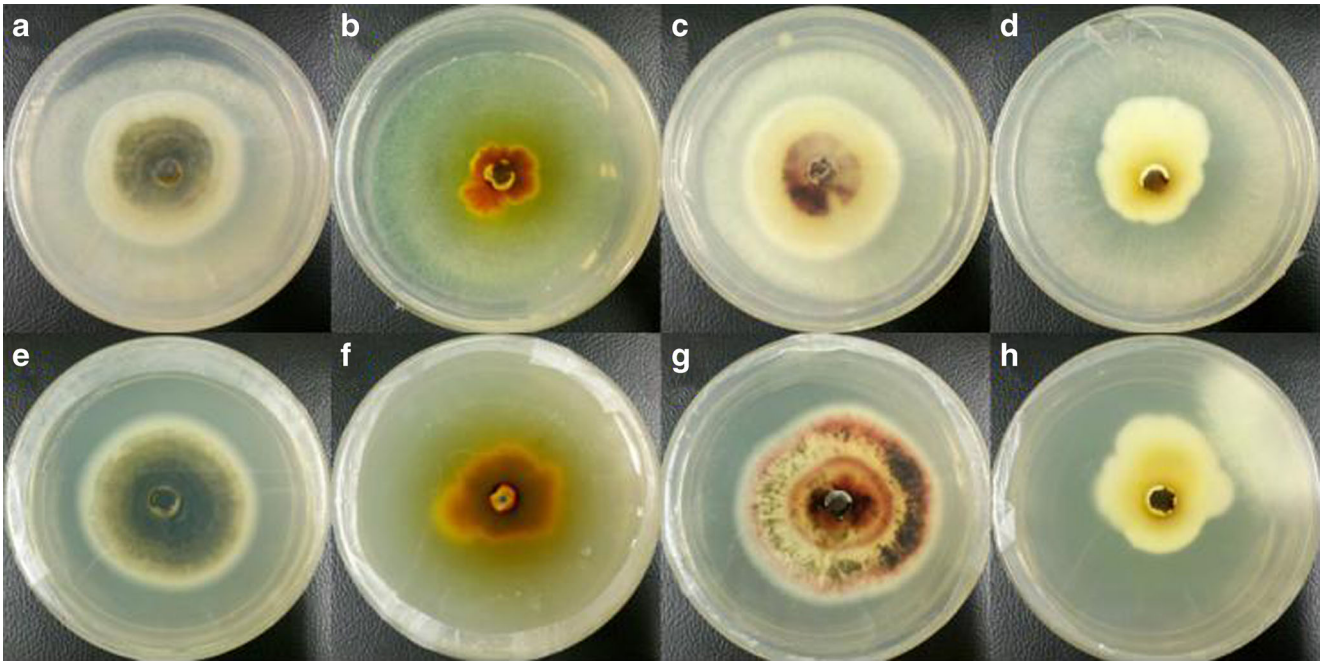

Fig. 5 Antagonism of VOCs produced by *T. koningiopsis* against four phytopathogens (recorded from 60 to 120 h). Two plates are shown together, phytopathogens on the upper and *T. koningiopsis* on the lower: **a** *S. lignicola*; **b** *E. nigrum*; **c** *Phoma herbarum*; **d** *F. flocciferum*; **e**, **f**, **g**, and **h** are the corresponding controls of **a**, **b**, **c**, and **d**, respectively

to coil around and inhibit the host hyphae development. In the case of *F. flocciferum*, *Trichoderma* formed obvious hockey shapes at the end of hyphal tips, to contact with the host hyphae. Therefore, the mycoparasitic activity of *T. koningiopsis* YIM PH30002 could be exerted by a direct attaching, coiling, and parallel growing alongside hyphae of the tested pathogens.

Antagonistic activity of VOCs produced by *T. koningiopsis* YIM PH30002

The endophytic *T. koningiopsis* isolate produced VOCs with different capacities to prevent the radial growth of the tested pathogens (Figs. 5 and 6). For *E. nigrum*, the percent inhibition was about 30–40 % within 60–120 h of observation, and

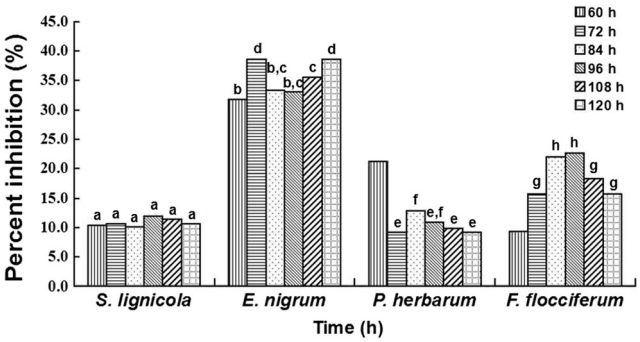

Fig. 6 Inhibition of four phytopathogens' mycelial growth by the *T. koningiopsis* antifungal volatile compounds on divided plates. Data were based on four replicates of the four phytopathogens. Means with the same letter for the same phytopathogen are not significantly different to each other according to Duncan's multiple range test, $P \leq 0.05$, $n = 4$

remained relatively stable (Fig. 6). The inhibition of *F. flocciferum* changed remarkably in the period from 60 to 120 h, and the highest inhibition was about 25 % at 90 h (Fig. 6). But in the cases of *S. lignicola* and *Phoma herbarum*, the inhibitions were not very effective (about 10 %), except reaching 20 % for *Phoma herbarum* at 60 h (Fig. 6). Continuous growth and new hyphae branches (data not shown) were observed after removal of the *T. koningiopsis* isolate.

Identification of VOCs

The GC-MS chromatography results showed that at least ten VOCs in the sample were produced by *T. koningiopsis* YIM PH30002 at 120 h inoculation in PDB medium (marked with capital letters A–M in Fig. 7a). They are identified and listed in Table 2, according to the match factor for sample spectra ≥90 % with reference to the NIST database. From the chromatogram, relative abundance ≥1.00 % included six kinds of volatile substances (Table 2) and alkenes made up more than 76 % abundance overall, among which cycloocta-2,4-dien-1-ol (Fig. 7a, peak J) was the greatest at 72.09 %.

Discussion

There are increasing reports about using *T. koningiopsis* as biocontrol agents (Mahalakshmi and Raja 2013). Saxena et al. (2015) reported that *T. koningiopsis* could mediate tolerance against biotic stress of phytopathogens in *Cicer*

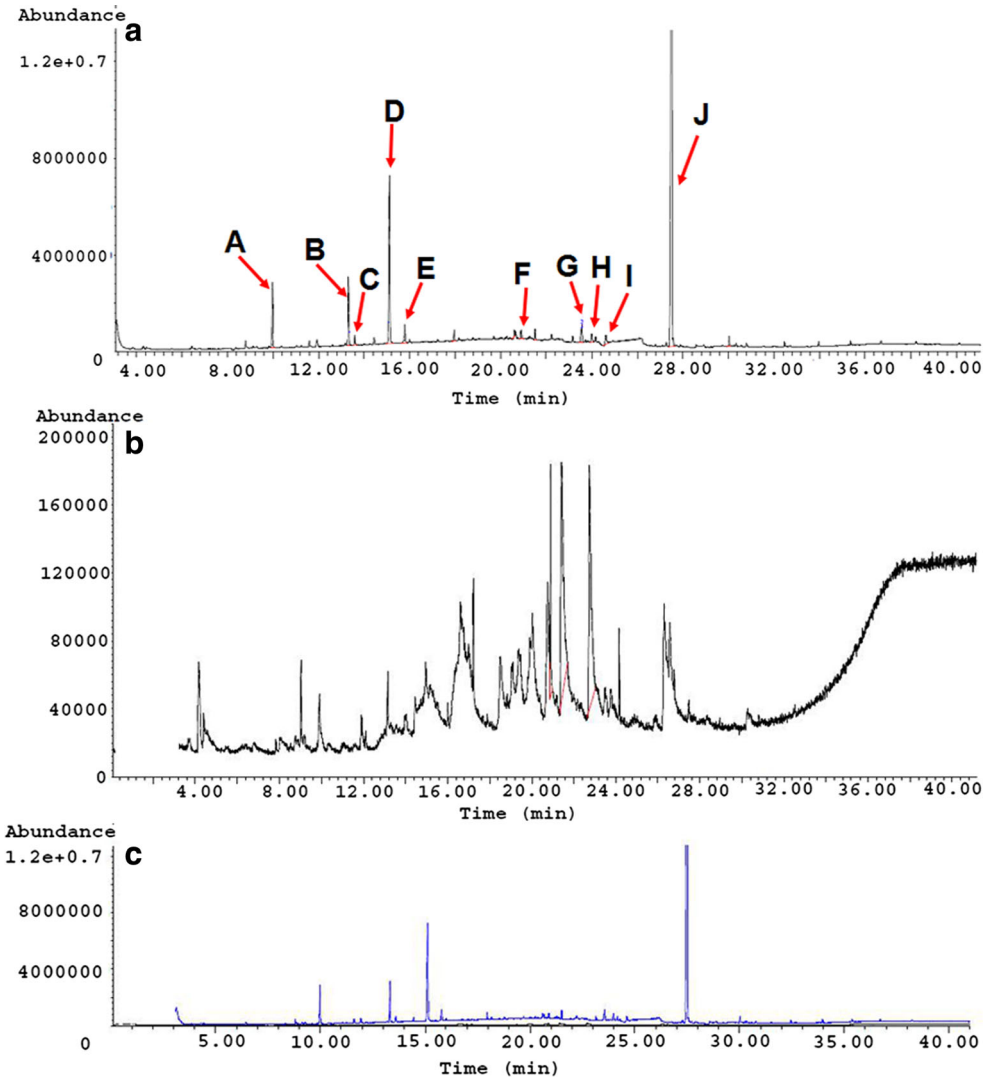

Fig. 7 The GC profile of volatile components: **a** the VOCs profile of *T. koningiopsis*; **b** the VOCs profile of the control (PDB medium); **c** the overlap spectrograms of *T. koningiopsis* (*blue line*) and the control (*black line*, not clearly indicated). The peaks of compounds produced by *T. koningiopsis* are recorded and identified in Table 2

arietinum. Moreno et al. (2009) found that *T. koningiopsis* could induce systemic resistance of tomato against *F. oxysporum* f. sp. *radicis-lycopersici* and establish its plant growth promotion ability. In this study, *T. koningiopsis* YIM PH30002 was confirmed as being harmless to the host plant of *P. notoginseng*.

Although no obvious antagonistic halo was observed in dual culture tests, *T. koningiopsis* YIM PH30002 grew quickly,

Table 2 VOCs of *T. koningiopsis* cultured in PDB extracted using SPME and identified by GC-MS

Retention time	Peak	Volatile compound	Relative abundance
9.976	A	β-Phellandrene	3.77
13.320	B	*trans*-1-Butyl-2-methylcyclopropane	3.78
13.598	C	2-Dodecene	0.68
15.118	D	*cis*-1-Butyl-2-methylcyclopropane	12.27
15.785	E	2-Tridecenal	1.36
21.509	F	Cyclohexene	0.2
23.567	G	1H-Cyclopropane	1.59
23.987	H	Phenanthrene	0.74
24.627	I	Anthracene	0.86
27.512	J	Cycloocta-2,4-dien-1-ol	72.09

covered the colonies and probably parasitized the hyphae, and inhibited the colony growth of the pathogens. A probable mycoparasitism of *T. koningiopsis* YIM PH30002 was microscopically observed as coiling, direct contact, and parallel growth alongside pathogens' hyphae. The mycoparasitic mode is typical in *Trichoderma* spp. (Aryantha and Guest 2006; Huang et al. 2011; Zhang et al. 2014), and has been proposed as the major mechanism accounting for antagonistic activity against the fungal pathogens, where the antagonist coils around the pathogen coupled with the production of lytic enzymes (Howell 2003; Harman et al. 2004). Widmer (2014) observed that *Trichoderma asperellum* could colonize a chlamydospore and sporangium of *Phytophthora ramorum*. *Trichoderma* spp. also can produce hydrolytic enzymes such as chitinase, β-1,3-glucanase, and protease, which can lyse and destroy the cell wall of fungal pathogens (Verma et al. 2007; Huang et al. 2011). High levels of cellulose, β-1,3 glucanase, and protease activity were correlated with strong biological activity of a *Trichoderma viride* isolate (Mishra 2010). We also found inhibition halos of tested fungi in agar diffusion tests (data not shown) with crude extracellular proteins secreted by *T. koningiopsis* YIM PH30002.

Fungal VOCs play an important role in the biocontrol of phytopathogens (Mercier and Jiménez 2009; Minerdi et al. 2009; Fialho et al. 2010, 2011), and exert signal molecules in the interactions of *Trichoderma* with plants and other microorganisms (Malmierca et al. 2015). VOCs are considered as a potential direct long-distance mechanism for antagonistic action to the pathogen *F. oxysporum*. Their antagonism could be the consequence of both the reduction of pathogen mycelial growth and the inhibition of pathogen virulence gene expression (Minerdi et al. 2009). In our bi-compartmented petri dishes tests, antibiosis caused by volatile metabolites of *T. koningiopsis* YIM PH30002 was clearly observed. Compared with the controls, pathogenic fungal colonial and pigment differences in the treatments (Fig. 5) revealed that volatile substances induced abnormalities in the hypha as well as metabolites. After removal of the *Trichoderma* isolate, the fungal pathogens continued to grow again, thus inferring that the VOCs are inhibitory to them, not fungicidal. The VOCs profile of *T. koningiopsis* YIM PH30002 was analyzed and identified by matching with the NIST database. At least ten volatile compounds were classified as alkanes (*trans*-1-butyl-2-methylcyclopropane, 2-dodecene, *cis*-1-butyl-2-methylcyclopropane, cyclohexene, cycloocta-2,4-dien-1-ol), monoterpenes (β-phellandrene) and arenes (phenanthrene, anthracene), heterocycles (1H-cyclopropane), and aldehydes (2-tridecenal). Noteworthy, β-phellandrene has favorable antagonism activity against pathogenic fungi and bacteria associated with many important crops (Al-Burtamani et al. 2005). In this report, we tested the VOCs produced by *T. koningiopsis* YIM PH30002 on PDB medium for the first time.

Panax notoginseng is usually planted in mountainous areas, with acidic soils (pH 4.5–5.5), high humidity (25–40 %), artificial shading, and amended with a large amount of chemical fertilizers, pesticides, and fungicides. These approaches may result in the disappearance of some *Trichoderma* spp. The isolation of *Trichoderma* species has been affected by environmental parameters such as soil temperature, moisture, pH, organic matter, and nutrient content present in the sampling site (Carreiro and Koske 1992; Klein and Eveleigh 1998). Meanwhile, soil physical and chemical parameters also influence *Trichoderma* activities, such as spore germination, germ-tube growth (Magan 1988), mycelial growth (Luard and Griffin 1981), and antagonistic properties (Tronsmo and Dennis 1978). Endophytes association fungi can resist salinity stress by producing gibberellins and activating defensive mechanisms of host plants to achieve improved growth (Khan et al. 2013). In contrast, biocontrol agents prepared with low osmotolerant *Trichoderma* species may lose their efficacy in protecting plants from pathogens with high salt concentration tolerance (Ragazzi et al. 1994; El-Abyad et al. 1988). However, *T. koningiopsis* YIM PH30002 presented higher osmotolerance against up to 27 % NaCl (w/v), with optimal salt concentration from 0 to 10 % NaCl, exhibiting appreciable resistance to abiotic stress.

Trichoderma koningiopsis YIM PH30002 was able to inhibit the growth of four phytopathogens from its host plant, *P. notoginseng*, by probable mycoparasitic mechanisms and producing volatile metabolites. The high osmotolerance properties refer that it can adapt and colonize in notoginseng planting soil with salinization by residual agrochemicals. All of the results suggest that the *T. koningiopsis* YIM PH30002 isolate has good potential to be a microbial agent for the biocontrol of *P. notoginseng* root-rot disease complex. Further studies should testify its efficacy to combat root-rot complex and to assist *P. notoginseng* in resisting biotic stress.

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