

Research Paper

## Antifungal activity of metabolites of the endophytic fungus *Trichoderma brevicompactum* from garlic

Xuping Shentu<sup>1,2</sup>, Xiaohuan Zhan<sup>2</sup>, Zheng Ma<sup>2</sup>, Xiaoping Yu<sup>2</sup>, Chuanxi Zhang<sup>1</sup>

<sup>1</sup>Institute of Insect Science, Zhejiang University, Hangzhou, China.

<sup>2</sup>College of Life Science, China Jiliang University,  
Zhejiang Provincial Key Laboratory of Biometrology and Inspection & Quarantine, Hangzhou, China.

Submitted: June 25, 2012; Approved: April 1, 2013.

---

### Abstract

The endophytic fungus strain 0248, isolated from garlic, was identified as *Trichoderma brevicompactum* based on morphological characteristics and the nucleotide sequences of ITS1-5.8S-ITS2 and *tefl*. The bioactive compound T2 was isolated from the culture extracts of this fungus by bioactivity-guided fractionation and identified as 4 $\beta$ -acetoxy-12,13-epoxy- $\Delta^9$ -trichothecene (trichodermin) by spectral analysis and mass spectrometry. Trichodermin has a marked inhibitory activity on *Rhizoctonia solani*, with an EC<sub>50</sub> of 0.25  $\mu\text{g mL}^{-1}$ . Strong inhibition by trichodermin was also found for *Botrytis cinerea*, with an EC<sub>50</sub> of 2.02  $\mu\text{g mL}^{-1}$ . However, a relatively poor inhibitory effect was observed for trichodermin against *Colletotrichum lindemuthianum* (EC<sub>50</sub> = 25.60  $\mu\text{g mL}^{-1}$ ). Compared with the positive control Carbendazim, trichodermin showed a strong antifungal activity on the above phytopathogens. There is little known about endophytes from garlic. This paper studied in detail the identification of endophytic *T. brevicompactum* from garlic and the characterization of its active metabolite trichodermin.

**Key words:** *Trichoderma brevicompactum*, garlic, endophytic fungus, Trichodermin, antifungal activity.

---

### Introduction

An increased concern over the widespread use of toxic agricultural chemicals and an increased recognition of the potential of biological pesticides has stimulated much research to develop and implement the use of biological pesticides (Epstein and Bassein, 2003; Strobel and Daisy, 2003). Chemical pesticides have been the objects of substantial criticism in recent years, mainly due to their adverse effects on the environment, human health and other non-target organisms (Raju *et al.*, 2003). The potential use of microbe-based biocontrol agents as replacements or supplements for agrochemicals has been addressed in many recent reports (Hynes and Boyetchko, 2006; Vasudevan *et al.*, 2002). Endophytes are microorganisms that live in the intercellular spaces of stems, petioles, roots and leaves of plants, and there is no discernible manifestation of their presence (Strobel and Long, 1998; Yang *et al.*, 2011). The

ymbiosis between plants and endophytes is well known; specifically, the former protects and feeds the latter, which 'in return' produces bioactive (plant growth regulatory, antibacterial, antifungal, antiviral, insecticidal, etc.) substances to enhance the growth and competitiveness of the host in nature (Carroll 1988; Konig *et al.*, 1999; Yang *et al.*, 1994). Accordingly, endophytes are currently considered to be a wellspring of bioactive secondary metabolites with the potential for medical, agricultural, and/or industrial exploitation (Schulz *et al.*, 2002; Tan *et al.*, 2000).

Garlic (*Allium sativum*), a member of the family Liliaceae, contains an abundance of chemical compounds that have antimicrobial, anticancer and antioxidant activity (Omar and Al-Wabel, 2010). However, little is known about the secondary metabolites of endophytes harbored inside the healthy garlic tissues. To find fungi for potentially efficient biopesticides, we isolated the endophytic

isolates from plants such as garlic and carried out a bioassay against phytopathogens. The candidate isolate 0248 showed strong inhibitory activity against phytopathogens. In this paper, the identification of the endophytic fungus isolate 0248 from garlic by morphological and molecular characteristics is described. The purification and characterization of antifungal metabolites from the fermentation broth of isolate 0248 are also studied.

## Materials and Methods

### Endophytic fungus and phytopathogenic strains

The endophytic fungus 0248 was isolated from healthy garlic based on antifungal activity. The phytopathogenic fungal strains, including *Fusarium oxysporum*, *Colletotrichum lindemuthianum*, *C. ampelinum*, *Rhizoctonia solani* and *Botrytis cinerea*, were kindly provided by the Institute of Plant Protection and Microorganisms, Zhejiang Academy of Agricultural Sciences. These strains were separately stored on PDA slants containing potato-dextrose agar at 4 °C until used.

### Culture conditions for isolate 0248

Fresh mycelium were picked and inoculated into 500 mL Erlenmeyer flasks containing 100 mL PD medium. After 2 days of incubation at  $28 \pm 1$  °C on a rotary shaker at 150 rpm, 3 mL culture liquid was transferred as seeds into a 300-mL Erlenmeyer flask containing 30 mL medium (20 gL<sup>-1</sup> dextrose, 5 gL<sup>-1</sup> peptone, 1 gL<sup>-1</sup> beef extract, 0.001 gL<sup>-1</sup> ZnSO<sub>4</sub>·7H<sub>2</sub>O and 0.01 gL<sup>-1</sup> NH<sub>4</sub>Cl). The resulting culture was kept on a rotary shaker at 180 rpm for 4 days at  $28 \pm 1$  °C. The fermentation broth was separated from the mycelia by filtration through filter paper using a Buchner funnel. One hundred milligrams mycelial biomass was taken following washing (two times) with sterile Tris-EDTA buffer for DNA extraction.

### Identification of the endophytic fungus isolate 0248

The candidate isolate 0248 was identified by using morphological and molecular procedures. The morphological examination was performed by observing the characteristics of fungal culture on PDA, yeast extract sucrose agar (YES) (40 gL<sup>-1</sup> yeast extract, 160 gL<sup>-1</sup> sucrose and 20 gL<sup>-1</sup> agar) and synthetic low-nutrient agar (SNA) (1.0 gL<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>, 1.0 gL<sup>-1</sup> KNO<sub>3</sub>, 0.5 gL<sup>-1</sup> MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.5 gL<sup>-1</sup> KCl, 0.2 gL<sup>-1</sup> dextrose, 0.2 gL<sup>-1</sup> Sucrose and 20 gL<sup>-1</sup> agar) in Petri dishes, separately. Microscopic slides were prepared and examined under a light microscope (Leica, German). Total genomic DNA was extracted from fresh mycelium according to the cetyltrimethylammonium bromide (CTAB, Sigma) procedure (Cappiccino and Sherman, 1996).

A region of nuclear rDNA, containing the internal transcribed spacer regions 1 and 2 and the 5.8S rDNA gene region, was amplified by polymerase chain reaction (PCR)

using the primer pair ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTA TTGATATGC-3') with the following protocol: 1 min initial denaturation at 94 °C, followed by 30 cycles of 1 min denaturation at 94 °C, 1 min primer annealing at 50 °C and 90 s extension at 74 °C, and a final extension period of 7 min at 74 °C. A fragment of *tef1* was amplified by the primer pair *tef1* fw (5'-GTGAGCGTGGTATCACCATCG-3') and *tef1* rev (5'-GCCATCCTGGAGACCAGC-3') with the following amplification protocol: 1 min initial denaturation at 94 °C, followed by 30 cycles of 1 min at 94 °C, 1 min at 59 °C and 50 s at 74 °C, and a final extension period of 7 min at 74 °C (Kraus *et al.*, 2004). PCR products were sequenced by Shanghai Invitrogen Co. (Shanghai, China). The DNA sequence obtained was submitted to GenBank and *TrichoKEY* for homology analysis by BLASTN and *TrichoKEY* 2 program, respectively. Phylogenetic trees were inferred using the neighbor-joining (NJ) algorithm (Saitou and Nei, 1987) from the PHYLIP suite programs (Felsenstein 1995). The topologies of the resulting unrooted trees were evaluated in a bootstrap analysis (Felsenstein 1985) based on 500 resamplings of the NJ dataset using the PHYLIP package.

### Analytical conditions

Silica gel (200-300 mesh) for column chromatography and GF<sub>254</sub> (30-40 μm) for TLC were produced by Qingdao Marine Chemical Factory, Qingdao, China. All other chemicals used in this study were of analytical grade. IR spectra were recorded in KBr disks on an FTIR-8400S instrument. All NMR experiments were performed on a Bruker AM 500 FTNMR spectrometer using TMS as the internal standard. Mass spectrometry (MS) data were obtained using an Agilent-5873 mass spectrometer at 70 eV.

### Antifungal assay

Antifungal activity was tested with the hyphae growth velocity assay (Lu *et al.*, 2000). The effective concentration of the sample causing a 50% inhibition of mycelial growth (EC<sub>50</sub>) was determined. The commercial antifungal agent carbendazim, produced by NingXia Sanxi Chemical Co., Ltd. China, was used as positive control. Mycelial discs (4 mm in diameter) of phytopathogenic fungi grown on PDA were cut from the margins of the colony and placed on the same medium containing different concentrations of the sample. A negative control was maintained with sterile water mixed with PDA medium. Each treatment had three replicates. The diameter of colony growth was measured when the fungal growth in the control had completely covered the Petri dishes. The inhibition percentage of mycelial growth was calculated as follows:

$$\text{Mycelial growth inhibition (\%)} = \frac{D_a - D_b}{D_a} \times 100$$

where  $D_a$  represents control colony diameter and  $D_b$  represents treated colony diameter. The colony diameter is in millimeters.

### Gas chromatographic analysis

The active compound was dissolved in HPLC grade methanol and quantified by GC (Agilent 6890N) with an HP-5 capillary column (5% phenyl methyl siloxane, 30 m x 320  $\mu\text{m}$  x 0.25  $\mu\text{m}$ ) with an FID detector and nitrogen as the carrier gas. The injector temperature was 250 °C, the detector temperature was 270 °C, the column temperature rose by program with an initial temperature of 100 °C maintained for 5 min and rising to 260 °C at a rate of 10 °C  $\text{min}^{-1}$  and maintained for 5 min. The flow rate of  $\text{N}_2$  was 1.5  $\text{mL min}^{-1}$ .

### Statistical analysis

All statistical analysis were performed using SPSS 15.0 software. The log dose-response curves allowed determination of the  $\text{EC}_{50}$  for the fungi bioassay according to probit analysis. The 95% confidence limits for the range of  $\text{EC}_{50}$  values were determined by the least-square regression analysis of the relative growth rate (% control) against the logarithm of the compound concentration (Rabea *et al.*, 2009).

## Results

### Identification of the endophytic fungus isolate 0248

On PDA medium, the isolate 0248 mycelium grew rapidly at  $28 \pm 1$  °C in darkness. The aerial mycelia were white to green and colorless on the reverse side of the plate. After 6 days at  $28 \pm 1$  °C conspicuous concentric rings were present, and colonies with a regular margin were 90 mm in diameter. No diffuse pigment or odor was noted. However, cultures of isolate 0248 grown on YES and SNA tended to form pustules and grew slowly. Colonies did not grow radially on SNA. This result might be due to component difference of PDA, SNA and YES. All colonies sporulated on PDA, SNA and YES medium at  $28 \pm 1$  °C in the dark. On PDA, conidiomata grew from stromata and consisted of single conidiophores that were grey-green, smooth, and oval to spherical. The diameter of conidiophores was 5.5–7.0  $\mu\text{m}$  (Figure 1).

The sequences of the region of ITS1-5.8S- ITS2 (GenBank accession number JN257218) and the fragment of *tef1* (GenBank accession number JN257219) were obtained in this study. The comparison of ITS1-5.8S- ITS2 and *tef1* genes of isolate 0248 with sequences in the GenBank database revealed that this endophytic fungus was highly homologous to different strains of *Trichoderma brevicompactum*, with nucleotide identities 99–100% for ITS1-5.8S- ITS2 and 97–100% for *tef1*. The topology of the phylogram in Figure 2 confirmed that 100% bootstrap val-

ues were detected between the ITS1-5.8S- ITS2 and *tef1* sequences of isolate 0248 and those of *T. brevicompactum*. Therefore, isolate 0248 was closely related to *T. brevicompactum*.

*TrichoKEY2* is a program for the quick molecular identification of *Hypocrea* and *Trichoderma* on the genus and species levels based on an oligonucleotide DNA BarCode (www.isth.info), a diagnostic combination of several oligonucleotides (hallmarks) specifically allocated within the internal transcribed spacer 1 and 2 (ITS1 and 2) sequences of the rRNA gene cluster (Druzhinina *et al.*, 2005). Identification of isolate 0248 using the oligonucleotide barcode showed that it belongs to the species *T. brevicompactum* and that the identification reliability was high.

### Extraction and purification of metabolites of the endophytic fungus

The isolation of active compounds from strain 0248 was carried out with a bioassay against *R. solani*, and the fractions with antifungal activity were used for further isolation. The fermentation broth (total volume 30 L) of isolate 0248 was collected and extracted three times with ethyl acetate (filtrate/ethyl acetate 1:1, v/v) at ambient temperature. A brown residue (8.25 g) was obtained by evaporating the solvent from the extract in a vacuum; the residue was separated by chromatography over a silica gel column (200 g, 100–200 mesh), eluting successively with 1000 mL each of petroleum ether/ethyl acetate (v/v, 30:1, 20:1, 10:1, 8:1, 6:1, 4:1, 2:1, and 1:1) and acetone to obtain 8 fractions (A1~A8). A5 was re-chromatographed over a silica gel column (50 g, 100–200 mesh) eluted with 500 mL each of petroleum ether/ethyl acetate (v/v, 20:1, 15:1, 10:1, 5:1, and 1:1), ethyl acetate and acetone, obtaining 4 fractions (4B1~4B4). Based on the TLC monitoring and antifungal test, 4B2 was further separated over a silica gel column (25 g, 100–200 mesh) eluted with 100 mL each of petroleum ether/ethyl acetate (v/v, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1 and 1:1) and acetone to give fractions 5C1~5C15. 5C9 and 5C10 were combined to yield 258 mg active compound T2.

### Structure elucidation of antifungal compound T2

Compound T2, which had strong antifungal activity, was identified as 4 $\beta$ -acetoxy-12, 13-epoxy- $\Delta^9$ -trichothecene (trichodermin) by spectral analysis, MS data and direct comparison with published information (Godtfredsen and Vangedal, 1965). Colorless crystal (pentane), mp 45–46 °C; +10.2° (*c* 0.1  $\text{mg mL}^{-1}$ ,  $\text{CHCl}_3$ ); IR:  $[\alpha]_D^{20}$  ( $\text{cm}^{-1}$ ) 2900, 1732, 1374, 1243 and 1080  $\text{cm}^{-1}$ ; The molecular formula of compound T2 was determined to be  $\text{C}_{17}\text{H}_{24}\text{O}_4$  by its spectral data ( $^1\text{H}$ ,  $^{13}\text{C}$  NMR and DEPT), MW 292.16746,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$ : 5.506(dd, 1H, J = 6.4, 3.2 Hz, H-4), 5.337–5.350(m, 1H, H-10), 3.757(d, J = 4.0 Hz, 1H, H-2), 3.546(d, 1H, J = 4.4 Hz, H-11), 3.059(d, 1H,



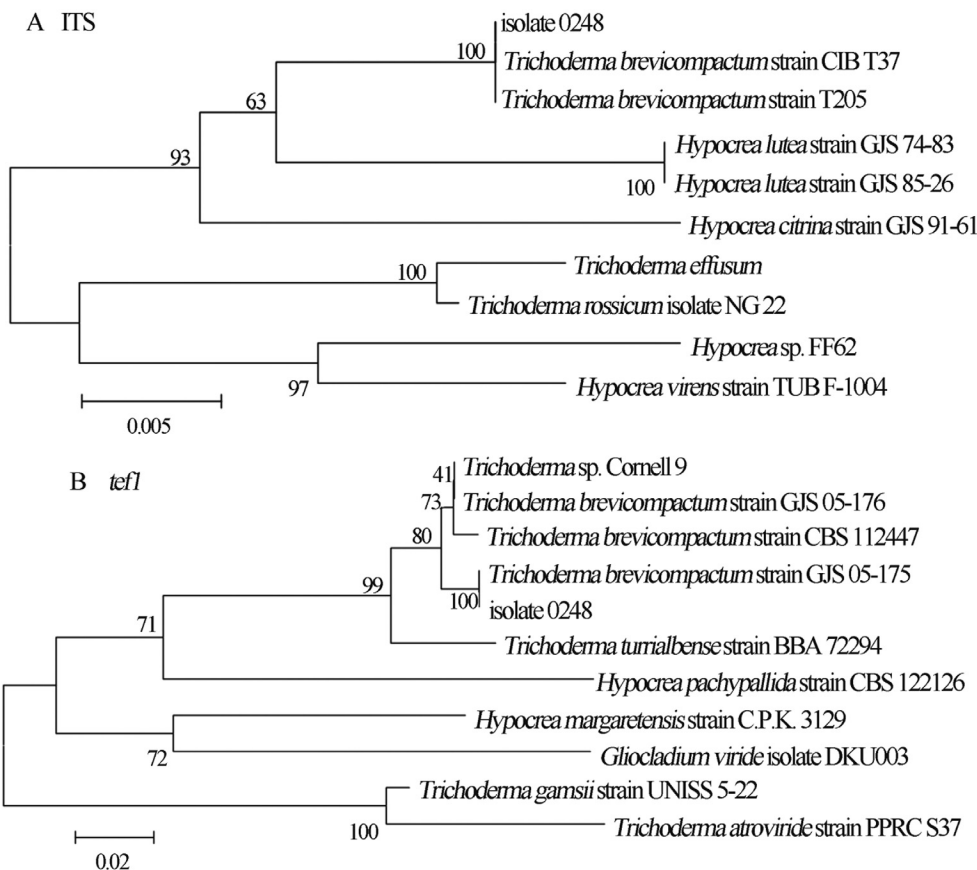


**Figure 1** - Morphology of isolate 0248. A, Appearance of colonies on YES; B, Appearance of colonies on PDA; C, Appearance of colonies on SNA; D, Appearance of pustules on SNA (arrow); E, Appearance of pustules on YES (arrow); F-I, hypha and conidia.

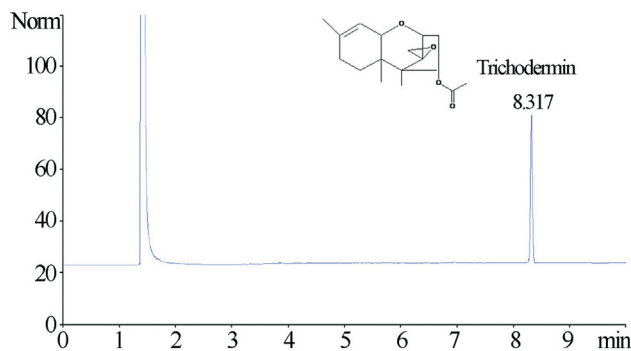
J = 3.2 Hz, H-13), 2.768 (d, J = 3.2 Hz, 1H, H-13'), 2.473(dd, J = 12.4, 6.4 Hz, 1H, H-3), 2.021(s, 3H, H-18), 1.951~1.865(m, 4 H, H-8, H-8', H-7, H-3'), 1.653 (s, 3H, H-16), 1.354(m, 1H, H-7'), 0.875(s, 3H, H-15), 0.658 (s, 3H, H-14).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$ : 170.71(C-17), 139.94(C-9), 118.42(C-10), 78.89(C-2), 74.86(C-4), 70.28(C-11), 65.30(C-12), 48.70(C-5), 47.62(C-13), 40.19(C-6), 36.44(C-3), 27.77(C-8), 24.24 (C-7), 23.06(C-16), 20.93(C-18), 15.78(C-15), 5.60(C-14). The spectrum of trichodermin used for quantitative analysis by GC is shown in Figure 3.

### Antifungal activities

The results of the antifungal activity of trichodermin against *R. solani*, *F. oxysporum*, *C. lindemuthianum*, *C. ampelinum* and *B. cinerea* were expressed as  $\text{EC}_{50}$  and are presented in Table 1. We observed that trichodermin markedly inhibits *R. solani*, with an  $\text{EC}_{50}$  of  $0.25 \mu\text{g mL}^{-1}$ . Trichodermin also strongly inhibits *B. cinerea*, with an  $\text{EC}_{50}$  of  $2.02 \mu\text{g mL}^{-1}$ . However, trichodermin had a poor inhibitory effect against *C. lindemuthianum* ( $\text{EC}_{50} = 25.60 \mu\text{g mL}^{-1}$ ). The results also indicated that the inhibitory effect of trichodermin differed with regard to the type of the fungi.



**Figure 2** - A Neighbor-joining tree based on ITS rDNA sequence of isolate 0248 and its closest ITS rDNA matches in GenBank. Values on the nodes indicate bootstrap percent confidence. B Neighbor-joining tree based on *tef1* sequence of isolate 0248 and its closest *tef1* matches in GenBank. Values on the nodes indicate bootstrap percent confidence.



**Figure 3** - Representative gas chromatography of trichodermin isolated and purified from fermentation broth by isolate 0248.

Compared with the positive controls, trichodermin has broad-spectrum and strong antifungal activity.

### Discussion

In our efforts to screen for suitable microorganisms from garlic that can produce bioactive compounds, we found that isolate 0248, subsequently identified as *T. brevicompactum*, showed strong antifungal activities. This is the first study of the endophytic fungus from garlic. *T.*

**Table 1** - Antifungal activities of trichodermin on mycelial growth of pathogenic fungi.

Microorganisms	EC <sub>50</sub> (µg mL <sup>-1</sup> )	
	Trichodermin	Carbendazim
<i>Rhizoctonia solani</i>	0.25	0.36
<i>Fusarium oxysporum</i>	8.51	10.35
<i>Colletotrichum lindemuthianum</i>	25.60	> 50.00
<i>Colletotrichum ampelinum</i>	16.65	> 50.00
<i>Botrytis cinerea</i>	2.02	> 50.00

*brevicompactum*, a new species, was first confirmed by morphological, molecular, and phylogenetic analyses in 2004, and related research has been very rare. *Trichoderma* species are common soil-borne fungi. One of the most significant ecological niches occupied by *Trichoderma* species is the plant rhizosphere (Harman *et al.*, 2004). The concept of *Trichoderma* as an endophyte has received a less attention.

Although *Trichoderma* isolates were isolated from live sapwood below the bark of trunks of wild and cultivated *Theobroma cacao* and other *Theobroma* species (Evans *et al.*, 2003), only a small number of *Trichoderma*

isolates from the collection have been studied for their biocontrol activities (Bailey *et al.*, 2008; Holmes *et al.*, 2004; Samuels *et al.*, 2006a, 2006b). However, it is unclear which compounds produced by these isolates have inhibitory activity against phytopathogens (Howell 2003). Purification of secondary metabolites from fermentation broths can be a challenging task due to the complexity of the medium, the inherent instability of the molecular structures or by the action of enzymes present in the fermentation broth, leading to poor isolation yield and loss of antibiotic activity. In this paper, we studied the isolation and purification of an active metabolite from the endophytic *T. brevicompactum* fermentation broth in detail and identified trichodermin as the main active compound.

It is well known that trichodermin is a very potent inhibitor of protein synthesis (Carrasco *et al.*, 1973). Reports of trichodermin activity against phytopathogens are less common. We studied the inhibitory activity of trichodermin against mycelial growth of plant pathogenic fungi and showed that it has potential value in agricultural applications. It was found that endophytic *T. brevicompactum* from garlic produced approximately  $86 \text{ mgL}^{-1}$  trichodermin in liquid cultures. This is a marked increase in trichodermin production compared with previous reports, in which *T. harzianum* Th008 produced approximately  $92.67 \text{ } \mu\text{gL}^{-1}$  trichodermin in liquid cultures of PDB (Bertagnolli *et al.*, 1998). Therefore, the high production of trichodermin and its high antifungal activity highlight the potential of this endophytic *T. brevicompactum* isolate in antibiotic production. In addition, very little was previously known about the nature of the relationship between *T. brevicompactum* and the garlic plant. Whether trichodermin has any influence on garlic growth is also an interesting possibility and should be researched in future.

## Acknowledgments

This work was supported by National Natural Science Foundation of China (30940018), Zhejiang Province Programs for Science and Technology Development (2010C32063, 2011R09027-03, and 2013C32008) and Zhejiang Province Natural Science Foundation (LY12C14012)

## References

- Bailey BA, Bae H, Strem MD, Crozier J, Thomas SE, Samuels GJ, Vinyard BT, Holmes KA (2008) Antibiosis, mycoparasitism, and colonization success for endophytic *Trichoderma* isolates with biological control potential in *Theobroma cacao*. *Biol Control* 46:24-35.
- Bertagnolli BL, Daly S, Sinclair JB (1998) Antimycotic compounds from the plant pathogen *Rhizoctonia solani* and its antagonist *Trichoderma harzianum*. *J Phytopathol* 146:131-135.
- Cappiccino JG, Sherman N (1996) *Microbiology a laboratory manual*, 6th ed. The Benjamin/Cummings Publishing Company, Redwood City, CA, USA.
- Carrasco L, Barbacid M, Vazquez D (1973) The trichodermin group of antibiotics, inhibitors of peptide bond formation by eukaryotic ribosomes. *Biochimica et Biophysica Acta (BBA) - Nucleic Acids and Protein Synthesis* 312:368-376.
- Carroll GC (1988) Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. *J Ecology* 69:2-9.
- Druzhinina I, Kopchinskiy AG, Komon M, Bissett J, Szakacs G, Kubicek CP (2005) An oligonucleotide barcode for species identification in *Trichoderma* and *Hypocrea*. *Fungal Genet and Biol* 42:813-828.
- Epstein L, Bassein S (2003) Patterns of pesticide use in California and the implications for strategies for reduction of pesticides. *Ann Rev Phytopathol* 41:351-355.
- Evans HC, Holmes KA, Thomas SE (2003) Endophytes and mycoparasites associated with an indigenous forest tree, *Theobroma gileri*, in Ecuador and a preliminary assessment of their potential as biocontrol agents of cocoa diseases. *Mycol Prog* 2:149-160.
- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783-791.
- Felsenstein J (1995) PHYLIP (Phylogenetic Inference Package), Version 3.75. Distributed by author. Department of Genetics, University of Washington, Seattle.
- Godtfredsen WO, Vangedal S (1965) Trichodermin, a new sesquiterpene antibiotic. *Acta Chem Scand* 19:1088-1102.
- Harman G, Howell CR, Viterbo A, Chet I, Lorito M (2004) *Trichoderma* species: opportunistic, avirulent plant symbionts. *Nat Rev* 2:43-56.
- Holmes KA, Schroers HJ, Thomas SE, Evans HC, Samuels GJ (2004) Taxonomy and biocontrol potential of a new species of *Trichoderma* from the Amazon basin in South America. *Mycol Prog* 3:199-210.
- Howell CR (2003) Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. *Plant Dis* 87:4-10.
- Hynes RK, Boyetchko SM (2006) Research initiatives in the art and science of biopesticide formulations. *Soil Biol Biochem* 38:845-849.
- Konig GM, Wright AD, Aust HJ, Draeger S, Schulz B (1999) Geniculol, a new biologically active diterpene from the endophytic fungus *Geniculosporium* sp. *J Nat Prod* 62:155-157.
- Kraus GF, Druzhinina I, Gams W, Bissett J, Zafari D, Szakacs G, Koptchinski A, Prillinger H, Zare R, Kubicek CP (2004) *Trichoderma brevicompactum* sp. nov. *Mycologia* 96:1059-1073.
- Lu H, Zou WX, Meng JC, Hu J, Tan RX (2000) New bioactive metabolites produced by *Colletotrichum* sp., an endophytic fungus in *Artemisia annua*. *Plant Sci* 15:67-73.
- Omar SH, Al-Wabel NA (2010) Organosulfur compounds and possible mechanism of garlic in cancer. *Saudi Pharm J* 18:51-58.
- Rabea EI, Badawy-Mohamed EI, Steurbaut W, Stevens CV (2009) In vitro assessment of N-(benzyl)chitosan derivatives against some plant pathogenic bacteria and fungi. *Eur Polym J* 45:237-245.

- Raju NS, Niranjana SR, Shetty H (2003) Effect of *Pseudomonas fluorescens* and *Trichoderma harzianum* on head moulds and seed qualities of sorghum. *Crop Improv* 30:6-12.
- Saitou N, Nei M (1987) The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406-425.
- Samuels GJ, Dodd S, Lu BS, Petrini O, Schroers HJ, Druzhinina I (2006) The *Trichoderma koningii* aggregate species. *Studies in Mycol* 56:67-133.
- Samuels GJ, Suarez C, Solis K, Holmes KA, Thomas SE, Ismaiel A, Evans HC (2006) *Trichoderma theobromicola* and *T. paucisporum*: two new species from South America. *Mycol Res* 110:381-392.
- Schulz B, Boyle C, Draeger S, Römmert AK, Krohn K (2002) Endophytic fungi: a source of novel biologically active secondary metabolites. *Mycol Res* 106:996-1004.
- Strobel G, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. *Microbiol Mol Biol R* 67:491-502.
- Strobel GA, Long DM (1998) Endophytic microbes embody pharmaceutical potential. *ASM News* 64:263-268.
- Tan RX, Meng JC, Hostettmann K (2000) Phytochemical investigation of some traditional Chinese medicines and endophyte cultures. *Pharm Biol* 38:22-32.
- Vasudevan P, Kavitha S, Priyadarsini VB, Babuje L, Gnanamanickam SS (2002) Biological control of rice diseases. In: Gnanamanickam SS, editor. *Biological Control of Crop Diseases*. New York. Basel: Marcel Dekker, Inc, pp 11-32.
- Yang SX, Gao JM, Zhang Q, Laatsch H (2011) Toxic polyketides produced by *Fusarium* sp., an endophytic fungus isolated from *Melia azedarach*. *Bioorg & Med Chem Lett* 21:1887-1889.
- Yang X, Strobel G, Stierle A, Hess WM, Lee J, Clardy J (1994) A fungal endophyte-tree relationship: *Phoma* sp. in *Taxus wallachiana*. *Plant Sci* 102:1-9.

All the content of the journal, except where otherwise noted, is licensed under a Creative Commons License CC BY-NC.