

## Potential inhibitors against *Sclerotinia sclerotiorum*, produced by the fungus *Myrothecium* sp. associated with the marine sponge *Axinella* sp.

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Received: 11 November 2007 / Accepted: 5 May 2008 / Published online: 4 June 2008  
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**Abstract** *Sclerotinia sclerotiorum* is a worldwide ascomycete fungal plant pathogen, which causes enormous yield losses on major economic crops such as crucifers, grain legumes and several other plant families. The objective of this research was to isolate and characterise some bioactive products from cultures of fungi associated with the marine sponge *Axinella* sp. In total, nine fungal isolates were obtained from the marine sponge *Axinella* sp. collected from the South China Sea. A group of test strains, including two G<sup>+</sup> strains (*Bacillus subtilis* and *Staphylococcus aureus*), two G<sup>-</sup> strains (*Escherichia coli* and *Pseudomonas aeruginosa*) and three fungi including two plant

pathogenic fungi *Sclerotinia sclerotiorum* and *Magnaporthe grisea* and *Saccharomyces cerevisiae*, were employed as the indicator organisms for bioactivity screening. Using antagonistic tests and bioactive screening of the ethyl acetate (EtOAc) extracts of the corresponding cultures, fungal isolate JS9 showed the stronger efficacy against the test indicator strains, especially the indicator fungal pathogens. Isolate JS9 was further identified as *Myrothecium* sp. by a combination of morphological features and 18S rDNA BLAST on GenBank. Two macrocyclic trichothecenes, roridin A (compound 1) and roridin D (compound 2) were purified by tracking the activity of the EtOAc extract fractions and characterised with spectral analyses including MS, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR and distortionless enhancement by polarization transfer (DEPT). *In vitro* antifungal tests showed that the two macrocyclic trichothecenes were bioactive against *S. cerevisiae*, *M. grisea* and *S. sclerotiorum* with minimal inhibitory concentrations of 31.25, 125 and 31.25 µg ml<sup>-1</sup> for roridin A, and 62.5, 250 and 31.25 µg ml<sup>-1</sup> for roridin D, respectively. The present investigation demonstrated that two antifungal trichothecenes including roridin A and roridin D produced by the fungus *Myrothecium* sp. isolated from the marine sponge *Axinella* sp. could be potential inhibitors against the plant pathogen *S. sclerotiorum*.

Lian Wu Xie and Shu Mei Jiang contributed equally to this work.

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**Keywords** *Sclerotinia sclerotiorum* · Roridin A ·  
Roridin D · *Myrothecium* sp. · *Axinella* sp. · Activity  
tracking method

## Introduction

*Sclerotinia sclerotiorum* is an ascomycete fungal pathogen that causes stem rot in crucifer crops (rapeseed, canola and mustard) and several other plant families such as grain legumes (soybean, bean and pea) and other oilseeds (sunflower), resulting in enormous yield losses all over the world (Bardin and Huang 2001; Pedras and Hossain 2007). This necrotrophic pathogen exhibits little host specificity and has a host range that includes >400 primarily dicotyledonous plant species (Boland and Hall 1994). Populations of *S. sclerotiorum* are predominantly clonal in temperate regions of North America, Europe and New Zealand (Hambleton et al. 2002). There is now evidence of an extensive epidemic of this pathogen in South America, Australia and Asia (Sexton et al. 2006; Wang et al. 2003). It is very crucial to find some efficient inhibitors to control the spread of this disease.

The mechanism of *S. sclerotiorum* pathogenicity is well understood after many molecular biological and proteomic studies (Hegedus and Rimmer 2005; Pedras and Hossain 2007; Yajima and Kav 2006). The highly effective inhibitors against *S. sclerotiorum* have been studied (Chrysayi-Tokousbalides and Kastanias 2003; Rodriguez et al. 2006) but an effective application has not been developed to date. At present, cultivation of resistant cultivars and biological control have become the two traditional ways to battle against *S. sclerotiorum* (Bardin and Huang 2001; del Rio et al. 2002; Heritage 2003). The third way is perhaps to find bioactive compounds or biological reagents to inhibit the growth and spread of the pathogen.

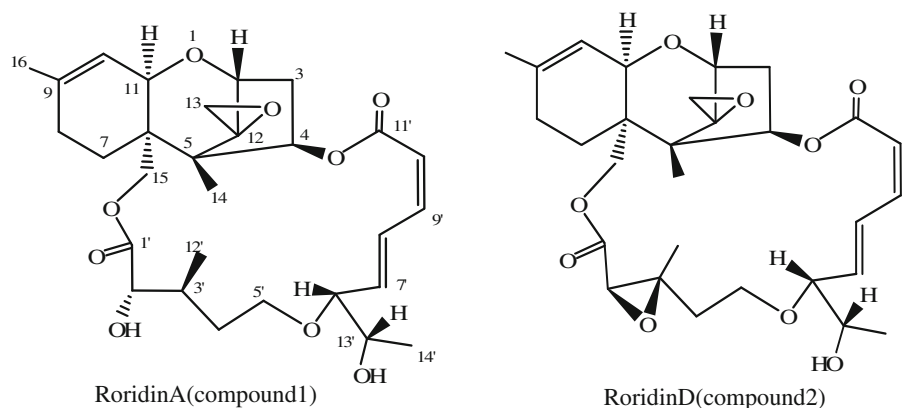
In our search for biologically potential natural products from marine sponges and associated microorganisms (Jiang et al. 2007), we observed that the EtOAc extract of a fungal isolate JS9 from the marine sponge *Axinella* sp., identified as *Myrothecium* sp., strongly inhibited the growth of *S. sclerotiorum*. The extract also inhibited the other two fungi, *Saccharomyces cerevisiae* and *Magnaporthe grisea*. Two active fractions of the EtOAc extract of the JS9 culture were purified by passing them through an open silica gel column, followed with Sephadex LH-20 column fractionation and preparative high-performance liquid chromatography to obtain two antifungal trichothecenes, roridin A and roridin D (see Scheme 1). Both compounds were strongly bioactive against *S. sclerotiorum*.

## Materials and methods

### General experimental procedures

Melting points were determined on a XRS-1 digital-melting point apparatus, and the values were uncorrected. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR data were collected on a Bruker AVANCE-500 (Bruker, Switzerland) spectrometer at 500 MHz, and the chemical shifts were recorded in  $\delta$  (ppm) relative to  $\text{Si}(\text{Me})_4$  with coupling constants  $J$  in Hz. Electron ionization-mass spectrometry (EI-MS) was performed using a MAT95XP mass spectrometer (Thermo, USA). Silica gel (100–200 mesh) for open column chromatography and prepared silica gel (300 mesh) plates for TLC were produced by the Qingdao Marine Chemical Factory (Qingdao, China). Sephadex™ LH-20 was from GE Healthcare

**Scheme 1** Structure of roridin A and roridin D



(GE, USA, made in Sweden). Ultimate purification of compounds was performed on a HITACHI L-2000 preparative high-performance liquid chromatography (HITACHI, Japan) with one YMC semi-preparative ODS column. All other chemicals used in this study were of analytical grade.

#### Isolation of strain

The fungal isolates JS1 to JS9 were obtained by a serial dilution method from sponge samples collected from the South China Sea (18° 13' N; 109° 29' E) near Sanya, Hainan, China in July, 2005. The sponge sample was later identified as *Axinella* sp. by Dr. K. J. Lee (Department of Biology, Hannam University, 133 Ojungdong, Daedukgu, Daejeon, Korea). The samples were kept in fresh seawater on ice and stored at -20°C until further analysis.

#### Culture media

The strain isolation was performed on the starch casein KNO<sub>3</sub> agar (SCKA) medium composed of 20 g starch, 2 g KNO<sub>3</sub>, 2 g K<sub>2</sub>HPO<sub>4</sub>, 2 g NaCl, 0.3 g casein, 0.05 g MgSO<sub>4</sub> 7H<sub>2</sub>O, 0.02 g CaCO<sub>3</sub>, 0.01 g FeSO<sub>4</sub> 7H<sub>2</sub>O, and 18.0 g agar in 500 ml sterile distilled water (SDW) premixed with 500 ml filtered sea water. The germination and growth of the marine fungal isolates were undertaken on the potato dextrose agar sea water (PDAS) medium, which was composed of 300 g diced potato, 20 g dextrose, 15 g agar in 500 ml SDW premixed with 500 ml filtered sea water.

#### Indicator strains

The initial bioactivity screening procedure involved fungal indicator organisms, as well as some Gram-positive (G<sup>+</sup>) and Gram-negative (G<sup>-</sup>) bacteria. The indicator strains included two plant pathogenic fungi *S. sclerotiorum* (ACCC 30046) and *Magnaporthe grisea* (*Pyricularia oryzae*) (ACCC 30320) from the Agricultural Culture Collection of China (ACCC) through professor Zanmin Hu of the Institute of Genetics and Developmental Biology, CAS. The two G<sup>-</sup> strains, *Escherichia coli* (GIM 1.115) and *Pseudomonas aeruginosa* (GIM 1.46), two G<sup>+</sup> strains, *Bacillus subtilis* (GIM 1.181) and *Staphylococcus aureus* subsp. *aureus* (GIM 1.178), and a common

yeast, *Saccharomyces cerevisiae* (GIM 2.86) were obtained from the Microbial Culture Collection Centre, Guangdong Institute of Microbiology.

#### Antagonistic test

For the fungal inhibition test, an inoculum plug (5 mm diam) of each isolate, prepared by punching the growth agar gel of a 72-h Petri dish culture, was transferred into the four corners of a 9 cm diam Petri dish containing PDAS medium. A plug of the fungal indicator organism (5 mm diam) was placed in the middle of the same Petri dish. The inoculated Petri dish was cultured at 28°C for 48 h. The antagonistic action was evaluated by measuring the diameter of the inhibition zones with fluconazole as a positive control. For the bacterial inhibition test, an indicator organism was spread on top of the Petri dish containing LB agar. An inoculum plug (5 mm diam) of each isolate, prepared by punching the growth agar gel of a 72-h Petri dish culture, was transferred into the four corners of the Petri dish with the indicator bacteria. The inoculated Petri dish was cultured at 28°C for 48 h. The antagonistic action was evaluated by measuring the diameter of the inhibition zones with fluconazole as a positive control. Each antagonistic test was performed in triplicate.

#### Fungal fermentation

The fresh mycelium of each of nine fungal strains grown on PDAS medium at 28°C for 5 days was inoculated respectively into 500 ml Erlenmeyer flasks containing 150 ml PDAS (without agar) medium. After 2 days of incubation at 28°C on a rotary shaker at 200 rpm, a 40 ml seed culture liquid was transferred into each 1000 ml Erlenmeyer flask containing 300 ml PDAS medium. The flasks were incubated for 10 days at 28°C and 200 rpm on a rotary shaker.

#### Antifungal tests of fungal fermentation extract

Each culture filtrate (5 l) was extracted four times with the same volume of EtOAc. After evaporation of the solvent from the combined extract *in vacuo*, the residue was used in an *in vitro* antifungal assay against the indicator fungi *S. sclerotiorum*, *M. grisea* and *S. cerevisiae* according to the methods described above.

Each EtOAc extract of the isolated fungus was tested in triplicate with fluconazole as a positive control.

#### Identification of the isolated strain

The fungal strain JS9 was identified as *Myrothecium* sp. by comparing its morphological features with the reference description (Nag Raj 1995). The strain JS9 was further identified to *Myrothecium* sp. by comparison of its 18S rDNA sequence with those submitted sequences on GenBank (<http://www.ncbi.nlm.nih.gov/>) via online BLAST analysis.

#### Thin plate chromatography (TLC) bioautography

The EtOAc extracts of each isolated fungus were spotted onto GF<sub>254</sub> silica gel plates (5 cm×2 cm) preloaded with fluorescent indicator, and developed by placing them in a glass chromatography tank containing 10 ml evenly mixed solution of CHCl<sub>3</sub>/MeOH (v/v, 10:1). Plates were removed from the tank as soon as the observable solvent front reached 4.8 cm in height. The dark spots on the dried plates visualised under UV radiation (254 nm) showed the presence of the UV-absorbing compounds. After the observed spots were marked with pencil, each of the dried plates (silicon gel side) was placed onto the surface of PDAS medium which was smeared smoothly with the test pathogenic microbe in advance. Preparation of the test fungi Petri dishes and drying of plates was done using gloves in a super-clean hood. Petri dishes containing plates were sealed with parafilm and kept at 28°C in a light incubator with a 12 h photoperiod. The inhibition zones were usually visible within 6 days, appearing as semi-transparent circular areas of decreased density. This TLC bioautography, combined with the activity tracking method, was used in this study to develop the purification procedure of the metabolites.

#### Separation and purification of metabolites

*Myrothecium* sp. was cultured in PDAS medium at 28°C and 200 rpm on a rotary shaker for 10 days. The culture broth (50 l) was filtered, and the filtrate was adjusted to pH 2.0 with 2.0 mol l<sup>-1</sup> HCl, before being extracted with EtOAc four times. The combined filtrates were concentrated *in vacuo*, and the obtained residue (15.2 g) was first fractionated by open column

chromatography on silica gel (petroleum ether-EtOAc gradient). The activity tracking method was applied throughout the separation and purification procedure. One active fraction (2.4 g), obtained by elution with 70% petroleum ether-EtOAc, was chromatographed again on a silica gel column (CHCl<sub>3</sub>-MeOH gradient). The active fraction (255 mg) eluted with 99% CHCl<sub>3</sub>-MeOH, was further chromatographed on a PHPLC equipped with a 10 mm×250 mm ODS column, and was eluted with 64% CH<sub>3</sub>OH-H<sub>2</sub>O. This fraction yielded 78 mg of compound 2 as colourless needle crystals from *n*-hexane. Another active fraction (3.2 g), obtained by elution with 60% petroleum ether-EtOAc, was chromatographed again on a silica gel column (CHCl<sub>3</sub>-MeOH gradient). The active fraction (347 mg), eluted with 95% CHCl<sub>3</sub>-MeOH was further purified on a Sephadex LH-20 column (CHCl<sub>3</sub>-MeOH, 1:1) and recrystallized from CHCl<sub>3</sub> to yield 109 mg of compound 1 as a colourless plate.

#### Bioassays and minimal inhibitory concentration detections of the purified compounds

Antifungal activities and minimal inhibitory concentration (MIC) values of compounds 1 and 2 (dissolved in dimethyl sulphoxide, DMSO) were assessed *in vitro* using test fungi *S. sclerotiorum*, *M. grisea* and *S. cerevisiae* by the agar serial dilution method described in the literature (Ter Laak et al. 1991), with DMSO as a negative control and fluconazole as a positive control. Each isolated compound of different concentration was performed in triplicate.

#### Characterizations and structure elucidations of metabolites

##### Roridin A (compound 1, see Scheme 1)

Colourless plate (CHCl<sub>3</sub>), mp. 197–203°C. EI-MS: 533 [M+H]<sup>+</sup>; <sup>1</sup>H-NMR data (CDCl<sub>3</sub>, 500 MHz): 3.85 (1H, d, *J*=5.0 Hz, H-2), 2.22(1H, ddd, *J*=4.7,4.7,15.3 Hz, H-3a), 2.46 (1H, dd, *J*=8.3, 15.4 Hz, H-3b), 5.78 (1H, m, H-4), 1.90 (2H, m, H-7), 2.03 (2H, m, H-8), 5.43 (1H, d, *J*=4.5 Hz, H-10), 3.59 (1H, m, H-11), 2.80 (1H, d, *J*=3.95 Hz, H-13a), 3.12 (1H, d, *J*=3.95 Hz, H-13b), 0.81 (3H, s, H-14), 4.44 (2H, s, H-15), 1.74 (3H, br.s, H-16), 4.10 (1H, s, H-2'), 2.01 (1H, m, H-3'), 1.78 (2H, m, H-4'), 3.54 (2H, m, H-5'), 3.65 (1H, m, H-6'), 6.00 (1H, dd, *J*=3.0, 15.5 Hz, H-7'), 7.66 (1H, dd, *J*=11.8, 15.4 Hz,

H-8'), 6.66 (1H, dd,  $J=11.3, 11.3$  Hz, H-9'), 5.80 (1H, d,  $J=11.5$  Hz, H-10'), 1.09 (3H, d,  $J=6.85$  Hz, H-12'), 3.62 (1H, m, H-13'), 1.19 (3H, d,  $J=6.1$  Hz, H-14');  $^{13}\text{C}$ -NMR data ( $\text{CDCl}_3$ , 500 MHz): 79.07 (C-2), 34.85 (C-3), 74.31 (C-4), 49.36 (C-5), 43.77 (C-6), 20.26 (C-7), 27.70 (C-8), 140.95 (C-9), 118.23 (C-10), 67.18 (C-11), 65.24 (C-12), 47.79 (C-13), 7.51 (C-14), 64.58 (C-15), 23.31 (C-16), 174.88 (C-1'), 75.58 (C-2'), 37.17 (C-3'), 33.06 (C-4'), 69.87 (C-5'), 84.05 (C-6'), 139.19 (C-7'), 126.10 (C-8'), 143.92 (C-9'), 117.55 (C-10'), 166.53 (C-11'), 14.77 (C-12'), 70.84 (C-13'), 18.25 (C-14'). These data are identical to those described by Breitenstein and Tamm (1975).

Roridin D (compound 2, see Scheme 1)

Colourless needle crystals ( $\text{CHCl}_3$ ), mp. 223–225°C. EI-MS: 531  $[\text{M}+\text{H}]^+$ ;  $^1\text{H}$ -NMR data (500 MHz): 3.85 (1H, d,  $J=5.1$  Hz, H-2), 2.18(1H, ddd,  $J=4.7, 4.7, 15.3$  Hz, H-3a), 2.46 (1H, dd,  $J=8.3, 15.4$  Hz, H-3b), 5.80 (1H, m, H-4), 1.95(2H, m, H-7), 2.05(2H, m, H-8), 5.44(1H, d,  $J=4.45$  Hz, H-10), 3.85(1H, m, H-11), 2.81(1H, d,  $J=3.95$  Hz, H-13a), 3.12(1H, d,  $J=4.0$  Hz, H-13b), 0.85(3H, s, H-14), 4.27(1H, d,  $J=12.4$  Hz, H-15a), 4.38(1H, d,  $J=12.4$  Hz, H-15b), 1.74 (3H, s, H-16), 3.31(1H, s, H-2'), 1.48(1H, m, H-4' $\alpha$ ), 1.81(1H, m, H-4' $\beta$ ), 3.63(2H, m, H-5'), 3.67(1H, m, H-6'), 5.97(1H, dd,  $J=3.2, 15.7$  Hz, H-7'), 7.53(1H, dd,  $J=11.6, 15.6$  Hz, H-8'), 6.60(1H, t,  $J=11.3$  Hz, H-9'), 5.84(1H, d,  $J=10.8$  Hz, H-10'), 1.62(3H, s, H-12'), 3.81(1H, m, H-13'), 1.20(3H, d,  $J=6.1$  Hz,

H-14');  $^{13}\text{C}$ -NMR data ( $\text{CDCl}_3$ , 500 MHz): 79.10 (C-2), 35.17 (C-3), 74.49 (C-4), 49.21 (C-5), 43.26 (C-6), 20.62 (C-7), 27.61 (C-8), 140.69 (C-9), 118.49 (C-10), 67.10 (C-11), 65.38 (C-12), 47.83 (C-13), 7.05 (C-14), 64.60 (C-15), 23.30 (C-16), 168.15 (C-1'), 63.26 (C-2'), 58.21 (C-3'), 39.65 (C-4'), 67.46 (C-5'), 85.75 (C-6'), 138.19 (C-7'), 126.37 (C-8'), 143.11 (C-9'), 118.19 (C-10'), 166.39 (C-11'), 17.44 (C-12'), 70.91 (C-13'), 18.22 (C-14'). These data are identical to those described by Breitenstein and Tamm (1975).

## Results and discussion

Several culture media including LB, SCKA, 2216E, PDA, YPD and PDAS were selected for germination and growth of the fungi isolated from the marine sponge *Axinella* sp. collected in the South China Sea near Sanya. As a result, the PDAS medium in 50% natural filtered sea water was the most suitable for marine fungal cultures.

Nine fungal strains numbered from JS1 to JS9 were isolated from *Axinella* sp. The antagonistic actions of these fungi are shown in Table 1, although nearly no antibacterial activity was detected when  $\text{G}^+$  strains *B. subtilis* and *S. aureus*,  $\text{G}^-$  strains *E. coli* and *P. aeruginosa* were used as test strains, but their antifungal actions as indicated by the antagonistic tests were fairly similar to those of EtOAc extracts

**Table 1** The antimicrobial activity of isolated fungi from the marine sponge *Axinella* sp

Strain no.	Antagonistic action							Antifungal action of EtOAc extract		
	$\text{G}^+$ bacteria		$\text{G}^-$ bacteria		Fungi			Sc	Mg	Ss
	Bs	Sa	Ec	Pa	Sc	Mg	Ss			
JS1	–	–	–	–	–	–	–	–	–	–
JS2	–	–	–	–	+	–	–	–	–	–
JS3	–	–	–	+	–	–	–	+	–	–
JS4	–	–	–	–	–	–	–	–	–	+
JS5	–	–	–	–	–	–	–	+	–	–
JS6	–	–	–	–	–	–	–	–	–	–
JS7	–	–	–	–	–	–	–	–	–	–
JS8	–	–	–	–	–	–	–	–	–	–
JS9	–	–	–	–	+	–	+	++	+	+++
Fluconazole	–	–	–	–	++	+	++	++	+	+

‘–’, no inhibition; ‘+’, ‘++’ and ‘+++’, the diameter of observed inhibition zones is in the ranges of 1–10, 11–20 and 21–30 mm respectively.

*Bs* *Bacillus subtilis*, *Sa* *Staphylococcus aureus* subsp. *aureus*, *Ec* *Escherichia coli*, *Pa* *Pseudomonas aeruginosa*, *Sc* *Saccharomyces cerevisiae* Hansen, *Mg* *Magnaporthe grisea*, *Ss* *Sclerotinia sclerotiorum*



derived from their corresponding cultures. The results of antimicrobial activity tests show that the fungus numbered JS9 produced the most effective antifungal metabolites. Moreover, the antifungal activity of the EtOAc extract of the JS9 culture was much stronger than that of fluconazole, which was used as a positive control under the same mass concentration (Table 1). The inhibition of the JS9 EtOAc extract was especially more effective against *S. sclerotiorum* than the other two test fungi *M. grisea* and *S. cerevisiae*. Subsequently, a scaled-up culture of JS9 was prepared to purify and characterise the antifungal metabolites.

Using the antifungal activity tracking method, two known metabolites (compound 1 and 2) were purified from the EtOAc extract of *Myrothecium* sp. JS9. Compound 1 was identified as roridin A (Böhner and Tamm 1966b; Breitenstein and Tamm 1975) and compound 2 was identified as roridin D (Böhner and Tamm 1966a; Breitenstein and Tamm 1975), by spectral analyses including MS, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR and DEPT.

The two purified fungal metabolites were bioassayed against test fungi *S. sclerotiorum*, *M. grisea* and *S. cerevisiae* by the serial dilution method (DMSO as solvent) and the MIC values are shown in Table 2. The results based on activity tracking and MICs test suggested that roridin A and roridin D are the major antifungal components in the fungal culture. Both compounds were strongly bioactive against the three test fungi, and especially against *S. sclerotiorum* with the MICs relatively lower than those of fluconazole used as a positive control in this study.

Because of its strong antifungal metabolites, strain JS9 was subjected to biological identification. The morphological features were recorded as follows. The fungus grew fast on both PDA and PDAS media, the colony attained 8–9 cm in diam after incubation in light at 28°C for 4 days. Aerial mycelia were white, floccose and concentrically scattered with large blackish wet drops, which were collected in a mass of mucous dark green conidia. The conidiomata were sporodochial, flat, and formed by densely branched and brush-like conidiophores. These features of strain JS9 were consistent with the description of the genus *Myrothecium* (Nag Raj 1995). This identification was further confirmed by comparison of the 18S rDNA sequence of strain JS9 (Access number: EU159531) with the data available in GenBank using online BLAST, and the homology to the 18S rDNA

sequences of *Myrothecium verrucaria*, *Myrothecium atroviride*, *Myrothecium leucotrichum* and *Myrothecium roridum* was 99%.

As we know, roridin A and roridin D belong to the macrocyclic trichothecenes class. Trichothecenes comprise a group of sesquiterpenes characterised structurally by the 12, 13-epoxy-trichothec-9-ene moiety. Very interestingly, it is reported that this sesquiterpene nucleus has been isolated both from fungal cultures like those of *Fusarium* spp. (Ishii and Ueno 1981; Kim et al. 1990), *Myrothecium* spp. (Abbas et al. 2002; Amagata et al. 2003) and *Stachybotrys* spp. (Sudakin 2003), and from some higher plants such as *Baccharis artemisioides* and *Baccharis coridifolia* (Habermehl et al. 1985; Rizzo et al. 1997), *Ficus fistulosa* and *Rhaphidophora decursiva* (Zhang et al. 2002). Biologically, macrocyclic trichothecenes were reported to be antileukemic (Jarvis et al. 1980, 1984), antimalarial (Isaka et al. 1999; Zhang et al. 2002), antimicrobial (Liu et al. 2006; Wagenaar and Clardy 2001), antiviral (Tani et al. 1995), phytotoxic and cytotoxic (Abbas et al. 2002; Masuda et al. 2007), and some were reported to possess animal toxicity (Hughes et al. 1989). In the present study, the two trichothecenes (compounds 1 and 2), obtained from the EtOAc extract of the fungus JS9 isolated from the marine sponge *Axinella* sp., were ascertained to be antifungal compounds with the MICs comparable with those of fluconazole.

In conclusion, this study demonstrated that the fungus *Myrothecium* sp. JS9, a symbiotic fungus in the marine sponge *Axinella* sp., was an efficient producer of antifungal trichothecenes including roridin A and roridin D. Both roridin A and roridin D are potentially useful inhibitors of *S. sclerotiorum*.

**Table 2** The MIC values of compounds 1 and 2 (in µg ml<sup>-1</sup>)

	Sc	Mg	Ss
Roridin A (1)	31.25	125	31.25
Roridin D (2)	62.5	250	31.25
Fluconazole <sup>a</sup>	125	250	125
DMSO (solvent)	–	–	–

‘–’ means no inhibition.

Sc *Saccharomyces cerevisiae* Hansen, Mg *Magnaporthe grisea*, Ss *Sclerotinia sclerotiorum*

<sup>a</sup> Fluconazole was co-assayed as a positive control

**Acknowledgements** This research was partially funded by the Knowledge Innovation Programme of the Chinese Academy of Sciences (KZCX2-YW-216, KZCX2-YW-211), and National Supportive Plan Project of Science and Technology (2006BAB19B02). Parts of this study were carried out at the Key Laboratory of Microbial Application and Innovative Technology of Guangdong Province as an open funding project. We also thank the financial support of the Hundred Talents Programme of the Chinese Academy of Sciences. Many thanks to Dr. K. J. Lee for identification of the marine sponge, to Professor Zanmin Hu for providing some of the indicator organisms, to Dr. Donna Smith for critical reading of this manuscript.

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