Diversity and antagonistic potential of Actinobacteria from the fungus-growing termite *Odontotermes formosanus*

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Abstract

43 Actinobacteria were isolated from the nest of *Odontotermes formosanus*. A phylogenetic analysis of 23 Actinobacteria isolates with different morphotypes showed that they did not form a monophyletic group. Antifungal bioassays exhibited that many strains inhibit both the termite cultivar *Termitomyces* and the competitor *Xylaria*. However, Actinobacteria inhibited the competitor *Xylaria* more severely than the termite cultural fungus *Termitomyces*. Furthermore, two Actinobacteria (*Streptomyces* sp. T33 and *S. bellus* T37) had a selective antifungal effect on *Xylaria*, with the inhibition zone of 25.5 and 8.9 mm, respectively. An actinomycin D was isolated from the strain T33 and had potent antifungal activity against *Xylaria* with IC_{50} value of less than 3.1 $\mu\text{g/mL}$. In addition, further bioassays showed that actinomycin D possessed potent antifungal activities against *Magnaporthe grisea* ($IC_{50}=0.9 \mu\text{g/mL}$), *Fusarium oxysporum* f. sp. *cucumerinum* ($IC_{50}=2.2 \mu\text{g/mL}$), *Valsa mali* ($IC_{50}=1.7 \mu\text{g/mL}$), *Rhizoctonia solani* ($IC_{50}=10.3 \mu\text{g/mL}$), *Dothiorella gregaria* ($IC_{50}=12.5 \mu\text{g/mL}$) and *F. oxysporum* f. sp. *mornordicae* ($IC_{50}=14.3 \mu\text{g/mL}$), which were comparable to those of referenced cycloheximide. The findings of the present study suggest that the termite-associated Actinobacteria have a potential to be used as microbial fungicide.

Keywords Diversity · Actinobacteria · *Odontotermes formosanus* · Actinomycin D · Antifungal activity

Introduction

Termites are fully eusocial and have potent ecological significance in the subtropics and tropics (Liu et al. 2013). The food from plant materials for termites is poor in nitrogen nutrition. One of the tactics to resolve this imbalance is to expand symbiosis with fungus, which has evolved with Macrotermitinae termites (Isoptera, Termitidae). These fungus-growing termites are mainly restricted to Asian and African ecosystems. Termite–*Termitomyces* association is obligate and mutualistic, with termites depending on the fungus for the degradation of plant material and as a nitrogen nutrient source, while the fungus achieves provisioning of plant material and a suitable microclimate for growth in the

termite nest (Poulsen 2015). When fungus-growing termites live in the comb of their nests, the mutualistic *Termitomyces* species is the dominant fungus in the nest; when the termites die, or the fungal comb is incubated without living termites in the laboratory, the fruiting bodies of the ascomycete *Xylaria* arise and rapidly cover the fungal comb (Visser et al. 2009). Thus, *Xylaria* may contend with *Termitomyces* for the nutritional substrate supplied by fungus-growing termites, thereby possibly having a negative impact on the productivity of cultivar *Termitomyces*. Termites are accordingly predicted to have special mechanisms to select the mutualistic *Termitomyces* or have evolved tactics to inhibit the antagonistic *Xylaria* within nests.

Actinobacteria are good potential defensive symbionts in fungus-growing termites because they are well-known antibiotic generators and occur as defensive microbes in other insect–fungus symbioses (Currie et al. 1999; Scott et al. 2008; Kroiss et al. 2010; Seipke et al. 2011). Termite nest had huge Actinobacteria diversity (Manjula et al. 2014) and housed more than 20% of Actinobacteria that could inhibit the growth of at least one tested organism (Sujada et al. 2014). It was also reported that some Actinobacteria from the fecal nest of active termites had antimicrobial activity

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against fungal entomopathogens of *Metarhizium anisopliae* (Chouvenec et al. 2013). Visser et al. had reported the antifungal activities of termite-associated Actinobacteria from African ecosystem against *Termitomyces* and *Pseudoxylaria* (Visser et al. 2012). But in the case of Asian fungus-growing termites, the potential role as defensive microbes of Actinobacteria has never been studied. *Odontotermes formosanus* in Asia is a fungus-growing termite. Here, the isolated Actinobacteria (43 isolates) were screened for their selective antibiotic effects against *Xylaria* using single *Xylaria* and *Termitomyces* strains. We report the species, specificity and potential of Actinobacteria isolated from the nest of *O. formosanus* as defensive microbes in this symbiosis.

Materials and methods

Sampling sites

Fungal comb of *O. formosanus* was collected in sterile Petri dish from the campus of Zhejiang Normal University (29°00'17.37"N, 119°29'54.84"E) in Jinhua city of Zhejiang Province, PR China, in August 2014.

Microbial isolation and identification

According to the methods detailed elsewhere (Poulsen et al. 2011) with some modifications, Actinobacteria were isolated from a fungal comb material in the nest of *O. formosanus*. Briefly, the fungal comb sample (about 0.1 cm³) was thoroughly fragmented and mixed in 700 µl of sterile water. The triturations were diluted in a tenfold dilution series, and aliquots of 100 µl from each dilution were spread on Gause's agar medium (consisting of 20 g of soluble starch, 1 g of KNO₃, 0.5 g of K₂HPO₄, 0.5 g of MgSO₄·7H₂O, 0.5 g of NaCl, 0.01 g of FeSO₄, and 20 g of agar in 1 L of distilled water). The medium was supplemented with 0.1 g L⁻¹ cycloheximide to suppress the fungal growth. They were incubated aerobically for 96 h at 28 °C. The pure actinomycetic colony from the appropriate dilution was streaked on new Gause's medium. The isolated strains were preserved on Gause's medium slants at 4 °C until used. Actinobacteria were identified by the 16S rRNA sequence compared with those in the Ribosomal Database Project (<https://rdp.cme.msu.edu/>).

Xylaria was isolated from hyphal stroma appearing on fragments of ~20 g of fungal comb that had been incubated for 7 days in a sealed glass cup. *Termitomyces* was isolated from the overground mushroom on the termite nest. *Xylaria* and *Termitomyces* were identified by the 5.8S rRNA sequence with those of referenced records (Pelaez et al. 2008; Rouland-Lefevre et al. 2002).

Screening bioassay

The antifungal screening bioassay of the Actinobacteria isolates against *Xylaria* and *Termitomyces* was carried out by the disc diffusion method (Visser et al. 2012). *Termitomyces* or *Xylaria* inoculum was grown in 250-mL Erlenmeyer flasks with 125 mL of MY (malt 2% and yeast 0.2% w/v) liquid broth for 4 days. The mycelia were collected and homogenized in demineralised water. 200 µL of the mycelial suspensions of *Termitomyces* or *Xylaria* was spread on MY agar (MYA) plates (diameter 90 mm). Actinobacteria were inoculated by putting a 5 × 5 × 5-mm cube of 3-week-old MYA cultures upside down on the *Termitomyces* or *Xylaria* plates. The diameters of the inhibition zones were measured, and average diameter values were calculated for each strain. All tests were performed in triplicate. Data are shown as mean values ± standard deviation. Statistical tests were performed in SPSS software (version 22) to test the selective antifungal hypothesis (Visser et al. 2012).

Microbial fermentation of Actinobacteria T33

Microbial fermentation of Actinobacteria T33 was performed as described (Zhang et al. 2013). The slant culture of strain T33 was inoculated in 150 mL Gause's liquid medium in a 500-mL Erlenmeyer flask, shaking at 180 rpm for 2 days at 28 °C. Afterwards, the inoculum was transferred as seed into 500-mL Erlenmeyer flask containing 150 mL of Gause's liquid medium and grown at 28 °C at an agitation of 180 rpm for 10 days.

Isolation and characterization of an active component of T33

The cultural broth (20 L) was extracted with ethyl acetate (4 × 20 L). The ethyl acetate extracts were combined and concentrated by evaporation of menstruum in vacuo. Then the crude extract (4.0 g) was chromatographed on silica gel (200–300 mesh) column eluting with CH₂Cl₂/MeOH mixtures of stably growing polarity (100:0, 100:1, 100:2, 100:4, v/v) affording four parts (F1: 2.0 g, F2: 1.0 g, F3: 0.3 g, F4: 0.2 g). The further purification of part 2 (CH₂Cl₂/MeOH, 100:1) by silica gave a pure compound **1** (800 mg).

Structural identification of the bioactive component **1** was made by mass and nuclear magnetic resonance (NMR) spectroscopies. The electrospray ionization mass spectrometry (ESI-MS) of the purified compound was recorded on a Mariner Mass 5304 instrument. ¹H NMR and ¹³C NMR spectra were measured in CDCl₃ with a Bruker AVANCE-400 (Bruker, Switzerland) spectrometer, and chemical shifts

were obtained as δ values (ppm) by referring to tetramethylsilane (TMS) and solvent signal as internal standards.

Antifungal activity of the metabolite

Antifungal assays were performed as described (Zhang et al. 2011, 2013) with a little modification. Purified compound 1 was dissolved in a volume of aqueous solution (composed of 1% DMSO and 4% Tween-80) to obtain the final concentration of 100 $\mu\text{g/mL}$. Solutions of serial concentrations of the compound were prepared by twofold serial dilution and mixed completely with liquid sterilized potato dextrose agar (PDA) in Petri dish (9 cm diameter). After transferring the mycelium of the tested fungi onto the center of solidified medium, the plates were incubated at $28 \pm 1^\circ\text{C}$ until the fungal mycelia reached the edges of the control plates. The antifungal indices were calculated as follows:

$$\text{Antifungal index \%} = (1 - D_a/D_b) \times 100,$$

where D_a is the diameter of the radial mycelium in the experimental plates (mm) and D_b the diameter of the radial mycelium in the control plates (mm).

Statistical analysis

Sequenced Actinobacteria were analyzed for specificity of geographical origins. A phylogenetic analysis was performed with the 16S rRNA sequences obtained from termite nest-related isolates, and the most closely relevant type strain for each isolate obtained from a type strain search in the Ribosomal Database Project (RDP), as well as the top hit for each of the isolates from a BLASTn search in GenBank (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Actinobacteria strains from Southern Pine beetles (Scott et al. 2008) (GenBank accession numbers EU798707-8) were also included. A neighbor-joining distance tree was conducted in MEGA 5.0 with 1000 bootstrap replicates (Visser et al. 2012).

Results and discussion

Diversity analysis of Actinobacteria in the nest of *O. formosanus*

43 Actinobacteria were isolated from the nest of *O. formosanus*. 23 Actinobacteria isolates with different morphotypes had their 16S rDNA gene amplified, which were deposited in GenBank (accession numbers KY213663-685). The phylogenetic analysis showed that Actinobacteria from the nest of *O. formosanus* did not form a monophyletic group (Fig. 1), which was consistent with the correlative results on termite from African ecosystem

(Visser et al. 2012). These Actinobacteria were mainly correlative with Actinobacteria from soil, as well as with Actinobacteria from dung beetle (Table 1). However, the sequenced Actinobacteria showed some specificity for geographical locations and were mainly isolated from Asia (frequently from China, Table 1).

It was reported that the Actinobacteria could be introduced to termite comb from surrounding soil (Makonde et al. 2015; Otani et al. 2016). Our isolated Actinobacteria were mainly relevant with Actinobacteria from soil. So it suggested that the nest Actinobacteria may come from the environmental soil. A comparison of the Actinobacteria diversities between the surrounding soil and the termite nest could be an important step towards understanding the situation of specificity with the *O. formosanus*. In addition, the isolates in our dataset were predominately characterized as *Streptomyces*. Frequent isolation of *Streptomyces* might be due to medium choice and culturing technique, and was unlikely to be indicative of the absence of other types of Actinobacteria from this symbiotic system (Hanshew et al. 2015). Indeed, other rare Actinobacteria, such as *Micromonospora*, have yet to be cultured in the comb of African termite (Visser et al. 2012).

Antibiotic effect of Actinobacteria on *Xylaria* and *Termitomyces*

The antifungal activities of the 43 Actinobacteria isolates against *Xylaria* and *Termitomyces* were performed by the disc diffusion method. 21 Actinobacteria that did not have inhibition zones were not shown. 20 Actinobacteria had antifungal activities against *Xylaria* and *Termitomyces*. Table 2 summarizes the antifungal effect of these Actinobacteria by showing values of inhibition zones. Generally, Actinobacteria inhibited *Xylaria* more severely than the termite cultural fungus *Termitomyces*. Furthermore, two Actinobacteria (*Streptomyces* sp. T33 and *S. bellus* T37) had a pronounced and exclusive antifungal effect on *Xylaria* strain, with the inhibition zone of 25.5 and 8.9 mm, respectively, and none had a strong influence exclusively on *Termitomyces*. Therefore, some Actinobacteria exert selective antifungal potential in the nest of *O. formosanus*.

The antifungal bioassays showed that many strains inhibit both the substrate competitor *Xylaria* and the termite cultivar *Termitomyces*, which matched with the previous Visser's report (Visser et al. 2012). However, contrary to Visser's results, we found that the competitor *Xylaria* was more susceptible to Actinobacteria than the termite cultural fungus *Termitomyces*. This difference could be attributed to the distinctive susceptibility of *Xylaria* or *Termitomyces* from different regions.

Phylogenetic tree showing the relationships between various *Streptomyces* species and strains, based on 16S rDNA sequences. The tree is rooted at the bottom left. Bootstrap values are indicated at the nodes.

Species and strains included (from top to bottom):

- T37
- T61
- T38
- KJ716536.1 *Streptomyces* sp.
- T16
- AB841019 *Streptomyces* sp.
- JF682781 *Streptomyces coeruleorubidus*
- GU991344 *Streptomyces* sp.
- AY999720 *Streptomyces coeruleus* (Type)
- AB184849 *Streptomyces bellus* (Type)
- T19
- EU931089 *Streptomyces* sp.
- T8
- KX279638 *Streptomyces* sp.
- AJ781321 *Streptomyces griseoincarnatus* (Type)
- T14
- EU841659 *Streptomyces variabilis*
- AJ781321 *Streptomyces griseoincarnatus* (Type)
- KX575853 *Streptomyces rochei*
- AB184237 *Streptomyces rochei* (Type)
- T60
- ATCC19812 *Streptomyces tendae* (Type)
- KM886202 *Streptomyces* sp.
- AB184326 *Streptomyces parvulus* (Type)
- T33
- T1
- KT443809.1 *Streptomyces* sp.
- AB184398 *Streptomyces pactum* (Type)
- EU798708 *Streptomyces* sp. (Southern Pine beetles)
- EU798707 *Streptomyces* sp. (Southern Pine beetles)
- AB184868 *Streptomyces hydrogenans* (Type)
- T21
- KU141350 *Streptomyces* sp.
- LC097195.1 *Streptomyces* sp.
- HQ607429 *Streptomyces malaysiensis*
- T55
- AB249918 *Streptomyces malaysiensis* (Type)
- EU603353 *Streptomyces* sp.
- T58
- T44
- EU595361 *Streptomyces malaysiensis*
- T17
- FJ532411 *Streptomyces malaysiensis*
- T64
- T9
- EU077190 *Streptomyces samsunensis* (Type)
- HQ607430.1 *Streptomyces malaysiensis*
- T45
- FJ626635 *Streptomyces* sp.
- KF772617 *Streptomyces* sp.
- DQ445794 *Streptomyces narbonensis* (Type)
- T25
- T40
- EU841619 *Streptomyces parvus*
- T10
- FJ481058 *Streptomyces lavendulae*
- JQ806215 *Streptomyces pratensis* (Type)

Scale bar: 0.005

Table 1 The sequenced Actinobacteria strains associated with *O. formosanus* included in the construction of the neighbor-joining tree: first BLAST hit of sequenced strains with strain name, ecological and geographical origins (if known) as well as the closest match to type strains from an RDP type strain search

Strain	1st BLAST hit GenBank	1st BLAST hit species name	EO	GO	RDP TSG	Type strain species name	Similarity (%)
T45	FJ626635	<i>Streptomyces</i> sp		China	JQ806215	<i>S. pratensis</i>	97.99
T14	EU841659	<i>S. variabilis</i>		China	AJ781321	<i>S. griseoincarnatus</i>	99.49
T33	KM886202	<i>Streptomyces</i> sp	Sea	China	AB184326	<i>S. parvulus</i>	98.11
T44	EU595361	<i>S. malaysiensis</i>		China	AB249918	<i>S. malaysiensis</i>	99.09
T21	KU141350	<i>Streptomyces</i> sp	Beetle	Korea	AB184868	<i>S. hydrogenans</i>	99.29
T17	EU603353	<i>Streptomyces</i> sp	Soil	Korea	AB249918	<i>S. malaysiensis</i>	98.73
T25	KF772617	<i>Streptomyces</i> sp	Bog	Algeria	DQ445794	<i>S. narbonensis</i>	99.16
T10	FJ481058	<i>S. lavendulae</i>	Soil	China	JQ806215	<i>S. pratensis</i>	99.93
T37	JF682781	<i>S. coeruleorubidus</i>	Soil	India	AB184849	<i>S. bellus</i>	99.22
T61	GU991344	<i>Streptomyces</i> sp	Soil	China	AB184849	<i>S. bellus</i>	99.15
T19	EU931089	<i>Streptomyces</i> sp	Soil	China	ATCC19812	<i>S. tendae</i>	99.78
T40	EU841619	<i>S. parvus</i>		China	JQ806215	<i>S. pratensis</i>	99.78
T8	KX279638	<i>Streptomyces</i> sp		Algeria	AJ781321	<i>S. griseoincarnatus</i>	100.00
T60	KX575853	<i>S. rochei</i>		India	AB184237	<i>S. rochei</i>	100.00
T16	AB841019	<i>Streptomyces</i> sp	Soil	Thailand	AY999720	<i>S. coerulescens</i>	99.49
T55	LC097195	<i>Streptomyces</i> sp		Thailand	AB249918	<i>S. malaysiensis</i>	100.00
T9	HQ607430	<i>S. malaysiensis</i>		China	EU077190	<i>S. samsunensis</i>	99.71
T1	KT443809	<i>Streptomyces</i> sp		China	AB184398	<i>S. pactum</i>	99.93
T38	KJ716536	<i>Streptomyces</i> sp	Soil	China	AY999720	<i>S. coerulescens</i>	99.56
T54	KP338102	<i>S. malaysiensis</i>	Soil	China	EU077190	<i>S. samsunensis</i>	99.78
T57	KJ744057	<i>Streptomyces</i> sp	Lake	Algeria	AB249918	<i>S. malaysiensis</i>	99.09
T58	HQ607429	<i>S. malaysiensis</i>	Soil	China	AB249918	<i>S. malaysiensis</i>	98.67
T64	FJ532411	<i>S. malaysiensis</i>	Soil	China	EU077190	<i>S. samsunensis</i>	98.66

EO ecological origin, GO geographic origin, RDP TSG RDP type strain GenBank

Table 2 Antifungal activity of Actinobacteria with zone of effect against *Xylaria* and *Termitomyces* (mm)

Strain	<i>Termitomyces</i>	<i>Xylaria</i>	Strain	<i>Termitomyces</i>	<i>Xylaria</i>
T59	15.5 ± 0.7	37.5 ± 6.8	T58	18.5 ± 0.7	30.0 ± 0.0
T17	16.0 ± 0.0	30.0 ± 0.0	T47	19.0 ± 1.4	25.0 ± 7.1
T55	12.0 ± 0.0	20.0 ± 0.0	T54	23.5 ± 2.1	20.0 ± 0.0
T51	13.0 ± 1.4	15.0 ± 0.0	T50	24.5 ± 0.7	44.0 ± 5.7
T56	24.5 ± 0.7	59.0 ± 0.7	T48	20.0 ± 0.0	29.0 ± 1.4
T15	17.5 ± 3.5	21.0 ± 2.8	T64	13.0 ± 0.7	29.0 ± 0.7
T31	17.0 ± 4.2	20.5 ± 0.7	T25	16.0 ± 5.7	15.0 ± 7.1
T57	25.5 ± 0.7	21.0 ± 0.0	T62	10.0 ± 0.0	20.5 ± 6.4
T9	14.0 ± 2.8	26.7 ± 4.6	T44	28.5 ± 0.7	20.0 ± 0.0
T30	22.0 ± 7.8	10.5 ± 0.7	T33	NI ^a	25.5 ± 0.7
T29	35.5 ± 0.7	30.0 ± 2.8	T37	NI	8.9 ± 0.7

NI not inhibited

Structure elucidation of the bioactive metabolite

Bioassay-guided separation of the ethyl acetate extract of *Streptomyces* sp. T33, which presented the best selectively

Fig. 2 Chemical structure of compound 1

antifungal activities against *Xylaria* and *Termitomyces*, afforded one active component. The bioactive metabolite was characterized by ESI-MS, NMR and comparisons with published data (Hollstein et al. 1975; Sajiday et al. 2011).

The structure was elucidated as actinomycin D (Fig. 2) based on the following evidences.

Actinomycin D reddish-orange powder; ESI-MS: m/z 1255 $[M+H]^+$ (calculated for $C_{62}H_{86}N_{12}O_{16}$ 1254.6285), 1277 $[M+Na]^+$; 1H NMR ($CDCl_3$) δ : 0.74 (6H, d, 5.6 Hz), 0.89 (6H, t, 7.2 Hz 9.0 Hz), 0.95 (6H, t, 5.4 Hz 6.3 Hz), 1.14 (6H, m), 1.25 (6H, s), 1.80 (3H, m), 2.07–2.24 (8H, m), 2.55 (3H, s), 2.67 (6H, m), 2.90 (12H, m), 3.54–3.72 (6H, m), 3.80 (1H, m), 3.95 (1H, m), 4.51 (1H, d, 6.6 Hz), 4.62 (1H, d, 6.2 Hz), 4.75 (2H, dd, 12.0 Hz 17.5 Hz), 5.18 (2H, m), 5.97 (2H, dd, 9.0 Hz 17.1 Hz), 7.16 (1H, d, 6.7 Hz), 7.36 (1H, d, 7.7 Hz), 7.63 (1H, d, 7.7 Hz), 7.75 (1H, d, 6.3 Hz), 8.01 (1H, d, 5.8 Hz), 8.17 (1H, d, 5.6 Hz); ^{13}C NMR ($CDCl_3$) δ : 7.8, 15.1, 17.4, 17.8, 19.0, 19.1, 19.1, 19.1, 19.2, 19.3, 21.6, 21.7, 22.9, 23.1, 26.9, 26.9, 29.7, 31.1, 31.5, 31.6, 31.8, 34.9, 34.9, 39.1, 39.3, 47.4, 47.6, 51.5, 51.5, 54.9, 55.2, 56.2, 56.3, 58.8, 58.9, 71.4, 71.5, 75.1, 75.1, 101.9, 113.5, 125.8, 127.6, 129.1, 130.3, 132.8, 140.5, 145.2, 146.0, 147.7, 166.3, 166.5, 166.5, 167.6, 167.7, 168.5, 169.0, 173.3, 173.3, 173.3, 173.7, 179.1.

Antifungal activity of the bioactive metabolite against *Xylaria* and other fungal pathogens

The antifungal activity of actinomycin D, treated with various concentrations against *Xylaria*, was determined in vitro as shown in Fig. 3. Actinomycin D had higher inhibition rate with an increasing concentration. The 50% inhibitory concentration (IC_{50} value) of actinomycin D was less than 3.1 $\mu g/mL$. Thus, actinomycin D showed pronounced inhibition activity against *Xylaria* and was the major contributor to selective antifungal activity of the T33 strain.

To confirm the selectivity of antifungal activity, the effect of actinomycin D against other seven phytopathogenic fungi was evaluated in vitro. The results (Table 3) showed that

Fig. 3 Antifungal activity of actinomycin D against *Xylaria*

Table 3 The IC_{50} values of compound actinomycin D against the tested phytopathogens ($\mu g/mL$)

Phytopathogens	Actinomycin D	Cycloheximide ^a
<i>M. grisea</i>	0.9 ± 0.0^A	2.3 ± 0.1^B
<i>V. mali</i>	1.7 ± 0.3^A	0.3 ± 0.0^B
<i>G. sanbinetti</i>	$>25 \pm 0.0^A$	3.3 ± 0.2^B
<i>R. solani</i>	10.3 ± 0.4^A	0.3 ± 0.0^B
<i>D. gregaria</i>	12.5 ± 0.8^A	0.9 ± 0.1^B
<i>F. oxysporum</i> f. sp. <i>cucumerinum</i>	2.2 ± 0.3^A	4.9 ± 0.2^B
<i>F. oxysporum</i> f. sp. <i>mornordicae</i>	14.3 ± 1.3^A	10.6 ± 1.0^B

Values represent the mean of three replications $\pm$ standard deviation

Means in the same row followed by the different superscript letters are significantly different ($P < 0.05$)

^aCycloheximide was co-assayed as a positive control

actinomycin D possessed considerable antifungal activities against *Magnaporthe grisea* ($IC_{50} = 0.9 \mu g/mL$) and *Fusarium oxysporum* f. sp. *cucumerinum* ($IC_{50} = 2.2 \mu g/mL$), which was more potent than the positive cycloheximide, with IC_{50} values of 2.3 and 4.9 $\mu g/mL$, respectively. Furthermore, its magnitude of antifungal activities against *Valsa mali* ($IC_{50} = 1.7 \mu g/mL$), *Rhizoctonia solani* ($IC_{50} = 10.3 \mu g/mL$), *Dothiorella gregaria* ($IC_{50} = 12.5 \mu g/mL$) and *F. oxysporum* f. sp. *mornordicae* ($IC_{50} = 14.3 \mu g/mL$) was comparable to referenced cycloheximide, with IC_{50} values of 0.3, 2.3, 0.9 and 10.6 $\mu g/mL$, respectively. However, it weakly inhibited the growth of *Gibberella sanbinetti* ($IC_{50} > 25 \mu g/mL$).

The insect/fungal/bacterial mutualism systems could potentially yield new and useful antifungal compounds (Kim et al. 2014; Sit et al. 2015; Van Arnem et al. 2016), which were from symbiont actinomycetes and utilized to maintain a monoculture fungal garden free from parasites (Haeder et al. 2009; Oh et al. 2009; Barke et al. 2010). However, few investigations have so far been carried out on any other ant-fungus symbiosis. Here, we found *O. formosanus*-associated actinomycete, *Streptomyces* sp. T33, had a selective antifungal effect on *Xylaria* strain and reveal that it was capable of generating selective antifungal actinomycin D. It was reported that the greater quantities of actinomycin D could inhibit the fungi *F. decemcellulare*, *Syncephalastrum racemosum*, and the black yeast *Phialophora fastigiata* (Schoenian et al. 2011). But this was the first report of antifungal activity against *Xylaria* for actinomycin D.

Conclusion

43 Actinobacteria were isolated from the nest of *O. formosanus*. A phylogenetic analysis showed that they did not form a monophyletic group and the Actinobacteria were mainly

relevant to Actinobacteria from soil. The bioassays showed that some nest-associated Actinobacteria contributed to the selectively antifungal activity within the nest of *O. formosanus*. A selectively antifungal actinomycin D was isolated from *Streptomyces* sp. T33. Future investigations are needed for a better understanding of the other antibiotics produced by other Actinobacteria in this system.

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Compliance with ethical standards

Conflict of interest There are no conflicts of interest to declare.

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