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ORIGINAL ARTICLE

Isolation and characterization of a new iturinic lipopeptide, mojavensin A produced by a marine-derived bacterium *Bacillus mojavensis* B0621A

Zongwang Ma[1,2], Nan Wang[1], Jiangchun Hu[1] and Shujin Wang[1]

Three lipopeptides were isolated by bioactivity-guided fractionation from the fermentation broth of *Bacillus mojavensis* B0621A. A new iturinic lipopeptide, named mojavensin A (1), was tentatively characterized by 1D, 2D NMR and MS spectroscopy, Marfey's method containing a novel peptide backbone of L-Asn_1, D-Tyr_2, D-Asn_3, L-Gln_4, L-Pro_5, D-Asn_6, L-Asn_7 and an *anteiso*-type of the saturated β-fatty acid side chain. Compound 2 and 3 were tentatively identified as *iso*-C16 fengycin B and *anteiso*-C17 fengycin B, respectively. These lipopeptides displayed dose-dependent antifungal activity against a broad spectra of phytopathogens and were weakly antagonistic to *Staphylococcus aureus*. Moreover, they all revealed cytotoxic activities against the human leukemia (HL-60) cell line. Mojavensin A, *iso*-C16 fengycin B, and *anteiso*-C17 fengycin B inhibited the growth of HL-60 with IC_{50} of 100, 100 and 1.6 μM, respectively.
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Keywords: *Bacillus mojavensis*; fengycins; iturin; mojavensin

INTRODUCTION

Most *Bacillus* species have the ability to produce three families of lipopeptides, iturins, fengycins and surfactins, which show versatile functions in the biological control of fungal phytopathogens.[1] Iturins, especially fengycins, have shown great potential in antagonistic activities *in vitro*.[2,3] Surfactins can enhance root colonization of beneficial *Bacillus subtilis* in the soil environment.[4] Moreover, fengycins and surfactins can also induce plant system resistance.[5,6] Plant-associated bacteria also have the potential to improve the growth of crops as plant growth-promoting rhizobacteria.[7,8] These lipopeptides have important roles and there may be some synergistic effects in the interaction of plants, microorganisms and phytopathogens in the soil ecosystem.

Marine microorganisms have become a research focus because of their special natural products and unique and extreme ecosystem habitats, although their isolation and culture remains a difficult problem.[9–11] Numerous new lipopeptides have been reported from marine habitat microorganisms recently. Cytotoxic mixirins A, B and C were isolated from a marine *Bacillus* sp.[12] Tauramamide antagonistic to *Enterococcus* sp. was isolated from marine *Brevibacillus laterosporus*.[13] Scopularides A and B possessing cytotoxic activities against pancreatic and colon tumor cell lines were isolated from a marine sponge-derived *Scopulariopsis brevicaulis*.[14] An antifungal fengycin derivative with 6-Abu residue was found from a culture of *Bacillus amyloliquefaciens* SH-B10.[15] Bacillomycin D is a new antifungal iturin isolated from a sponge-derived *B. subtilis*.[16] Mitsoamide revealing cytotoxic activities against NCI-H460 human lung tumor cells was isolated from a marine cyanobacterium *Geitlerinema* sp.[17]

In the present investigation, a potential biological control agent, *Bacillus mojavensis* B0621A, was isolated from *Pinctada martensii* in the South China Sea. In contrast, the type strain of *B. mojavensis* RO-H-1 was isolated from desert soil environment[18] Lipopeptides produced by this strain were isolated, purified by HPLC system, elucidated by NMR spectroscopy and MS spectrometry techniques, and the antimicrobial activities were evaluated with paper disc–agar diffusion assay.[19] The cytotoxic activities were based on 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay.[20]

RESULTS AND DISCUSSION

Compound **1** (22.6 mg) was purified from the fraction from vacuum flash chromatography (VFC; dichloromethane/methanol, 0 : 100, v/v) using the semi-preparative HPLC system described above, eluting with 75% methanol containing 0.05% trifluoroacetic acid (v/v)) at 17.35 min. Compound **2** (25.0 mg) and **3** (30.0 mg) were purified from the fraction of VFC (dichloromethane/methanol, 70 : 30, v/v) using semi-preparative HPLC eluents of 80% methanol containing

[1]Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, China and [2]Graduate University of Chinese Academy of Sciences, Beijing, China
Correspondence: Professor J Hu, Institute of Applied Ecology, Chinese Academy of Sciences, 72 Wenhua road, Shenyang 110016, China.
E-mail: hujc@iae.ac.cn
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0.05% trifluoroacetic acid (v/v) at 29.81 min and 42.17 min, respectively.

Structure elucidation

Mojavensin A (**1**) was obtained as a white amorphous powder having a molecular formula of $C_{50}H_{77}N_{13}O_{14}$ as found in the HR ESI MS spectrum, (see Supplementary Figure S7) which showed a $[M+H]^+$ ion peak at *m/z* 1084.5674 (calcd for 1084.5791) and a $[M+Na]^+$ ion peak at *m/z* 1106.5605 (calcd for 1106.5611), respectively. The ^{1}H NMR (600 MHz, DMSO-d_6) spectrum (see Supplementary Figure S1) showed 17 N-bonded protons (δ 8.67–6.86) and seven α-protons (δ 4.63 −4.00) of peptide bonds, one *para*-substituted benzene ring (δ 6.65, 7.01, each d, J = 8.4Hz, 2H), one long fatty acid chain (δ 1.42 −1.06), and two terminal methyl see groups (δ 0.84, m, 6H). The ^{13}C NMR (150 MHz, DMSO-d_6) spectrum (see Supplementary Figure S2)showed the presence of 13 carbonyl groups (δ 170.5 −174.6), one *para*-substituted benzene ring (δ 155.9, 129.9, 129.9, 127.9, 127.9, 115.2), α-carbons of seven amino acids (δ 61.0 −49.9), methylene carbons (δ 47.4 −22.7), and two methyl carbons (δ 19.2, 11.4), as well as the shape of two terminal methyl groups (δ 0.84, m, 6H) of the ^{1}H NMR spectrum, indicating that the fatty acid chain was *anteiso*-type.[21,22] Seven amino acids, including Asn (4 ×), Gln (1 ×), Pro (1 ×) and Tyr (1 ×), were identified on the basis of the ^{1}H-^{1}H COSY (see Supplementary Figure S3), HSQC (see Supplementary Figure S4) and HMBC (see Supplementary Figure S5) experiments. The sequence of the amino-acid residues was determined by the ^{1}H-^{1}H COSY, HMBC and ROESY (see Supplementary Figure S6) experiments, showing that that **1** contained the following sequence of amino acids: Asn_1, Tyr_2, Asn_3, Gln_4, Pro_5, Asn_6, Asn_7 and β amino acid with–$(CH_2)_9(CH_3)CH_2CH_3$ group as a side chain (Figure 1). The absolute configuration of the fatty acid chain of iturin A was determined to be R by CD spectrum [23], whereas the configuration of the stereocenter in the fatty acid side chain was not determined in this study. From comprehensive consideration of all the results of **1**, including analysis of Marfey's derivatives, the stereochemistry of these amino acids could be assumed as follows: L-Asn_1, D-Tyr_2, D-Asn_3, L-Gln_4, L-Pro_5, D-Asn_6, L-Asn_7, respectively. In conclusion, **1** was a new compound belonging to the iturin family with amino acids composition of four Asn, and was named mojavensin A (Figure 1). The full assignment of ^{1}H and ^{13}C NMR chemical shifts of *anteiso*-C15 mojavensin A were based on the ^{1}H-^{1}H COSY, HSQC, HMBC and ROESY experiments (Table 1).

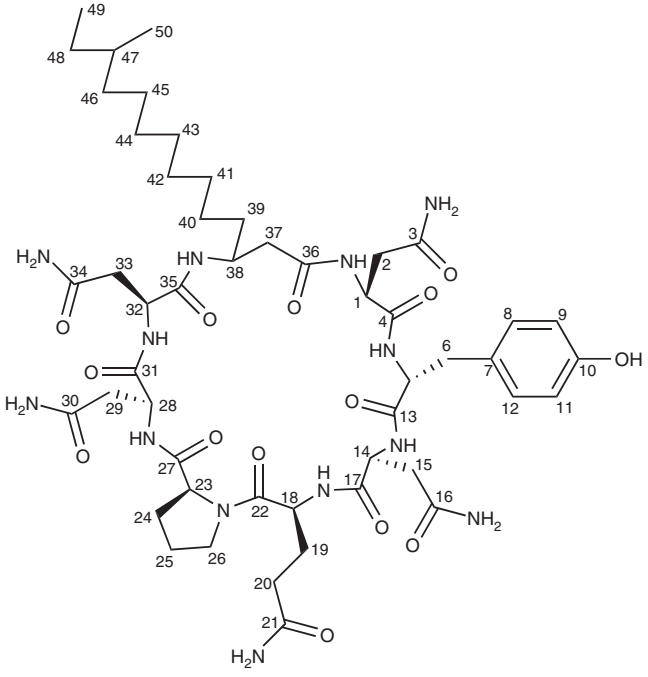

Figure 1 The structure of *anteiso*-C15 mojavensin A (**1**).

The ^{13}C NMR (150 MHz, DMSO-d_6) data indicated that the fatty acid chain of **2** and **3** were *iso*- and *anteiso*-type, respectively (Supplementary Figure S8 and Figure S11).[21] The full scan spectrum (MS1) of **2** showed singly and doubly-charged molecular ions at *m/z* 1491.99 $[M+H]^+$ and *m/z* 746.42 $[M+2H]^{2+}$, respectively (Supplementary Figure S9). The MS1 of **3** also showed singly and doubly-charged molecular ions at *m/z* 1506.05 $[M+H]^+$ and *m/z* 753.44 $[M+2H]^{2+}$, respectively (Supplementary Figure S12). The doubly-charged molecular ion was chosen as the precursor ions for further tandem MS fragmentation. The fragments mainly contained b- and y-type ions (Figure 3 and Figure 4). The base peaks of **2** and **3** were *m/z* 1108.63 and 1108.67 [M-fatty acid-Glu]$^+$, respectively (Supplementary Figure S10 and Figure S13). Both **2** and **3** had the same b-type ions but different y ions, showing that the two compounds shared the same amino acids sequence of Glu_1, Orn_2, Tyr_3, Ile_4, Tyr_5, Gln_6, Pro_7, Val_8, Glu_9, Thr_{10}, but different fatty acid side chains. The stereochemistry of **2** and **3** were determined by Marfey's method successfully as follows: L-Glu_1, D-Orn_2, D-Tyr_3, L-Ile_4, L-Tyr_5, L-Gln_6, L-Pro_7, D-Val_8, L-Glu_9, D-Thr_{10}, respectively. Compound **2** was identified as *iso*-C16 fengycin B, whereas **3** was *anteiso*-C17 fengycin B (Figure 2). The specific enantiomers of asparagine in **1** and of tyrosine in **2** and **3** have been positioned solely by analogy with the known members of these families.

Antimicrobial and cytotoxic activity of three lipopeptides

In this study, the potential anti-microorganism activities and the cytotoxicity to tumor cell lines of the three lipopeptides were tested. The three lipopeptides all showed activities to soil-borne phytopathogens and slight inhibition to *Staphylococcus aureus*, but no activity to *Candida albicans*. Moreover, they all showed weak cytotoxic activities against the human leukemia (HL-60) cell line. Mojavensin A (**1**), *iso*-C16 fengycin B (**2**) and *anteiso*-C17 fengycin B (**3**) inhibited the growth of HL-60 with IC_{50} of 100, 100 and 1.6 μM, respectively.

Iturins, especially fengycins, display outstanding antibiotic activities. *Anteiso*-C15 mojavensin A (**1**) exhibited dose-dependent antifungal activity when the concentration was over 2 mg ml^{-1} for *Valsa mali*, *Fusarium oxysporum* f. sp. *cucumerinum*, and *Fusarium verticillioides*. *Iso*-C16 fengycin B (**2**) and *anteiso*-C17 fengycin B (**3**) demonstrated obvious concentration-dependent antifungal activities to the targeted filamentous phytopathogens (Table 2). Beyond the present knowledge, the antifungal activity of *anteiso*-C15 mojavensin A (**1**) was not as excellent as the other iturins, and the MIC (2 mg ml^{-1}) of *anteiso*-C15 mojavensin A (**1**) was larger than for other iturins. Both the length of fatty acid chains and the different branches of iturins affected their antifungal activity.[22] The structure–activity relationship of mojavensin A remains unclear and required further investigation. Two fengycins (**2** and **3**) had stronger antifungal abilities than iturin (**1**) over the results of this study.

Generally, *F. verticillioides* is a endophytic phytopathogen that produces fumonisin mycotoxins and has been recognized as a threat to maize, maize-containing products and other crops.[24] A Leu^7-surfactin antagonistic to *F. verticillioides* has also been reported.[25]

Table 1 ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data assignments for mojavensin A (1) measured in DMSO-d_6[a]

Moiety	*Position*	δ_C	δ_H *(J in Hz)*	^{1}H-^{1}H *COSY*	*Selected HMBC*	*Selected ROESY*
Asn_1	1	50.5	4.41 m	2	2, 4	2
	2	36.7	2.20 dd (5.4, 6.0)/2.29 m		1, 4, 3	
	3	171.1[b]				
	4	173.3				
	1-NH		7.79 d (5.4)	1	1	1, 38
	3-NH_2		6.91 s/7.28 s		3	
Tyr_2	5	56.6	4.00 m	6		6
	6	35.2	2.73 m/2.91 dd (4.7, 4.6)		5, 7, 8, 12	
	7	127.9				
	8, 12	129.9	7.00 d (7.8)			9, 11, δ_H 9.16
	9, 11	115.2	6.64 d (8.4)			8, 12, δ_H 9.16
	10	156				
	13	171.4				
	5-NH		8.61 d (6.0)	5	6	1, 5
Asn_3	14	50.8	4.40 m	15	17	15
	15	36.2	2.49 m/2.55 dd (9.0, 9.0)		14, 16, 17	
	16	171.4				
	17	171.4				
	14-NH		8.05 d (7.2)	14	13, 14, 15	5, 14
	16-NH_2		6.86 s/7.21 s		16	
Gln_4	18	49.9	4.44 m	19		19, 20, 26
	19	26.4	1.88 m/1.94 m		20	
	20	30.8	2.12 m		21, 28	
	21	174.6				
	22	171.1[b]				
	18-NH		7.17 d (7.8)	18	18, 19	14, 18
	21-NH_2		6.87 s/7.27 s		21	
Pro_5	23	61	4.14 t	24		24
	24	29.3	1.74 m/2.11 m	23	24, 25, 27	
	25	24.7	1.86 m/1.96 m	24		
	26	47.4	3.68 m/3.82 m	25	25	
	27	173				
Asn_6	28	50	4.32 m	29	29, 31	29
	29	35.6	2.47 m/2.66 d (4.8)		30	
	30	172				
	31	170.5				
	28-NH		8.66 d (7.8)	28	27, 28, 29	23, 28
	30-NH_2		6.83 s/7.36 s		30	
Asn_7	32	50.4	4.63 m	33	33	33
	33	37.7	2.25 m/2.68 d (4.9)		32, 34	
	34	171.1				
	35	171.1				
	32-NH		7.32 d (8.4)	32		28, 32
	34-NH_2		6.88 s/7.18 s		34	
fatty acid	36	171.3[b]				
	37	42.2	2.31 m	38		38
	38	46.1	3.94 m	39		
	39	34.7	1.41 m	40		
	40	25.6	1.12 m/1.21 br s	41		
	41–44	28.8–29.3	1.22 m			
	45	26.7	1.22 m			
	46	36.2	1.05 m/1.14 m		45, 50	
	47	33.9	1.21 br s	48	46	
	48	29.1	1.06 m/1.27 m	49	46, 47, 49, 50	
	49	11.4	0.80 m			
	50	19.2	0.83 m	47		
	38-NH		7.10 d (9.0)	38	38, 39	32, 37, 38, 39

[a]The full assignments of ^{1}H and ^{13}C NMR chemical shifts of *anteiso*-C15 mojavensin A were based on ^{1}H-^{1}H COSY, HSQC, HMBC and ROESY experiments.
[b]Values may be interchangeable within the same column.

Fumonisin, especially fumonisin B_1, can increase the incidence of neural tube defects and inhibit eukaryotic protein synthesis, and it can also be a potential threat to human health.[26,27] *In vitro* antifungal experiments indicated that *anteiso*-C15 mojavensin A (**1**) and the two fengycins (**2** and **3**) had dose-dependent antagonistic activity against *F. verticillioides* and other phytopathogens. The interrelationships between fumonisin and lipopeptides should be further investigated.

The crude fingerprint of the lipopeptides (Supplementary Figure S15) indicated that *B. mojavensis* B0621A could secrete iturins, fengycins and surfactins. Surfactins produced by this strain had no antifungal activity after bioactive testing, but they may have had a critical role in the colonization of the bacteria in the soil–rhizosphere system. Our study has shown that *B. mojavensis* B0621A is a potential biological control agent, which can be applied in sustainable agricultural and crop protection.

METHODS

General experimental procedures

A semi-preparative HPLC system (Dionex U3000, Sunnyvale, CA, USA) was used in this study for analysis and further purification. One-dimensional and two-dimensional NMR spectroscopy (^{1}H-NMR, ^{13}C-NMR, ^{1}H-^{1}H COSY, HSQC, HMBC, ROESY) were conducted with a Bruker AV600 spectrometer (Leipzig, Germany, 600 and 150 MHz for ^{1}H and ^{13}C NMR, respectively), using DMSO-d_6 (δ_H 2.49 and δ_C 39.6, respectively) as internal references. The HR ESI MS experiments were performed with Bruker micro Q-TOF MS (Germany) and the Waters Micromass Q-TOF 2 (Manchester, UK) MS experiment was used to determine the sequence of the amino acids composition.

Strains and fermentation conditions

The *B. mojavensis* B0621A was isolated from the mantle of a pearl oyster *P. martensii* collected from Weizhou Island (21°05′N, 109°11′E) in the South China Sea. The strain was identified by morphological, physiological and biochemical characteristics and the sequencing of 16S rDNA (see Supplementary Figure S14; GenBank accession number: JN585825).[18] The culture conditions were based on Liu's medium[28] with some modification as follows: sucrose 2.0%, NH_4NO_3 0.2%, KH_2PO_4 0.3%, Na_2HPO_4 1.0%, $MgSO_4 \cdot 7H_2O$ 0.02%, yeast extracts 0.02%, $CaCl_2$ 0.7 μg, $MnSO_4 \cdot 4H_2O$ 1 μg, and pH 7.0–7.2. The *B. mojavensis* B0621A was cultured in several 250-ml flasks with 50 ml of medium at 28 °C for 20 h, and were then used as the seed culture. The seed culture was then inoculated into 3-l flasks each containing 1.5 l of medium, and cultured for 48 h at a stirring rate of 180 r.p.m.

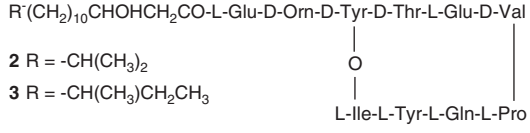

Figure 2 The structure of *iso*-C16 fengycin B (**2**) and *anteiso*-C17 fengycin B (**3**).

Extraction, isolation and purification of lipopeptides

The fermentation broth (24 l) of *B. mojavensis* B0621A was adjusted to pH 8.0 with 4 N NaOH and then centrifuged at 4000 *g* for 30 min. The supernatant was then acidified to pH 2.0 with 6 N HCl and kept overnight at 4 °C. The precipitate was collected by centrifugation at 4000 *g* for 30 min, and rinsed with HCl (pH 2.0) three times, followed by extraction of the residue with methanol three times. Finally, the solvent was evaporated under reduced pressure to yield 15.51 g of crude lipopeptides. The crude lipopeptides were eluted by dichloromethane/methanol (eight different ratios) using VFC over silica gel (600 ~ 800 mesh). Activity-guided fractionation and purification were conducted in this study using *Fusarium oxysporum* f. sp. *cucumerinum* as the targeted phytopathogen. Paper disc–agar diffusion assay established by Raahave is an efficient and simple method to evaluate the antibiotic activity.[19] The antifungal fractions from VFC were further purified by a semi-preparative HPLC system using C18 YMC-Pack ODS-A column (5 μm, φ 10 × 250 mm) at a flow rate of 2.5 ml min^{-1} with UV detection at 220 nm.

Absolute stereochemistry of amino acid residues

As described for Marfey's method,[29] lipopeptides (1 mg) were hydrolyzed with 6 N HCl (1 ml) at 110 °C for 24 h, and were then dried in a vacuum. The hydrolyzes were added to H_2O (50 μl), 1% (w/v) FDAA (Marfey's reagent, 100 μl) in acetone and 1 M $NaHCO_3$ (20 μl). The mixture was heated at 37 °C for 1 h, after which 2 M HCl (10 μl) was added and the mixture was then evaporated to dryness. The residue was dissolved in H_2O : DMSO (v/v, 1 : 1) (2 ml) and analyzed by reversed-phase HPLC (Luna 5UC18 Column, Phenomenex Inc., Torrance, CA, USA, φ 4.6 × 250 mm, UV detection at 340 nm, flow rate of 1 ml min^{-1}) using a linear gradient (10% acetonitrile containing 0.05% trifluoroacetic acid (v/v) to 50% acetonitrile within 60 min). The final solution was analyzed by coinjection with standard L-/D-amino acids and compared with the Marfey's derivatives of authentic amino acids.

In vitro antimicrobial spectrum and the cytotoxic activities determination

Stock solutions were prepared by dissolving the purified lipopeptides in methanol to a concentration of 1 mg ml^{-1}. Working solutions were made by twofold serial dilution of the stock solutions with methanol to test concentrations. Paper disc–agar diffusion assay was used to test the antimicrobial

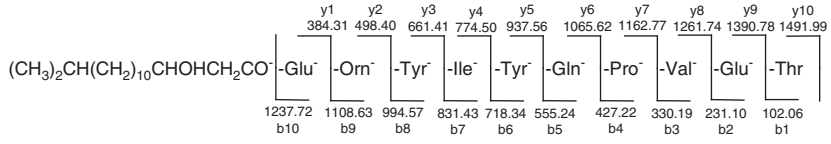

Figure 3 Positive ESI-Q-TOF MS2 fragmentation of *iso*-C16 fengycin B (**2**) resulting from precursor ion of *m/z* 1491.99 $[M+H]^+$ and 746.42 $[M+2H]^{2+}$.

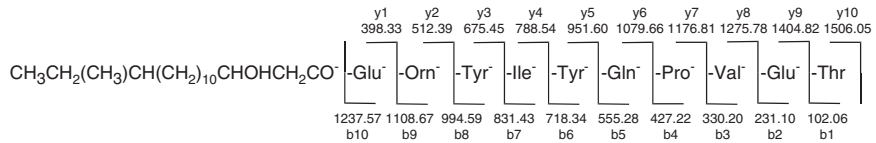

Figure 4 Positive ESI-Q-TOF MS2 fragmentation of anteiso-C17 fengycin B (**3**) resulting from precursor ion of *m/z* 1506.05 $[M+H]^+$ and 753.44 $[M+2H]^{2+}$.

Table 2 ***In vitro*** **antifungal spectra of** ***iso*****-C16 fengycin B (2) and** ***anteiso*****-C17 fengycin B (3)**

		Inhibition zone (mm) [a,b]							
		Concentration of ***2*** *(mg ml^{-1})*				*Concentration of* ***3*** *(mg ml^{-1})*			
Phytopathogen (PSA, 28°C)	*Time*[c]	*0.125*	*0.250*	*0.500*	*1.000*	*0.125*	*0.250*	*0.500*	*1.000*
Valsa mali	60	8.31 ± 0.24	8.87 ± 0.36	9.69 ± 0.32	10.49 ± 0.30	6.57 ± 0.08	7.50 ± 0.43	9.30 ± 1.15	10.43 ± 0.20
Fusarium oxysporum f. sp. *cucumerinum*	60	6.93 ± 0.36	7.03 ± 0.43	8.15 ± 0.14	8.99 ± 0.39	6.99 ± 0.54	7.45 ± 0.57	7.99 ± 0.41	9.09 ± 0.61
Fusarium oxysporum f. sp. *vasinfectum*	60	—	7.20 ± 0.26	7.73 ± 0.20	9.77 ± 0.88	6.24 ± 0.21	7.13 ± 0.53	8.06 ± 0.03	8.50 ± 0.35
Fusarium oxysporum f. sp. *vasinfectum.* SF 2	72	6.14 ± 0.18	6.79 ± 0.18	7.70 ± 0.09	9.77 ± 0.88	6.25 ± 0.34	6.78 ± 0.33	8.07 ± 0.18	8.55 ± 0.25
Fusarium solani SF 130	60	5.43 ± 0.16	7.19 ± 0.13	7.85 ± 0.52	9.84 ± 0.47	6.07 ± 0.19	7.83 ± 0.97	8.78 ± 0.43	9.98 ± 0.50
Botryosphaeria berengriana f. sp. *piricola*	48	7.08 ± 0.29	8.37 ± 0.42	9.49 ± 0.50	10.10 ± 0.27	7.17 ± 0.11	7.44 ± 0.34	8.49 ± 0.24	8.98 ± 0.59
Botrytis cicrea	60	8.75 ± 0.62	10.18 ± 0.33	10.71 ± 0.21	11.88 ± 0.47	7.95 ± 0.64	8.90 ± 0.24	9.85 ± 0.50	11.65 ± 1.06
Rhizoctonia solani J. G. Kühn	24	—	—	6.65 ± 0.26	8.37 ± 0.20	—	—	—	6.63 ± 0.54
Fusarium solani	60	5.65 ± 0.34	8.47 ± 0.38	9.19 ± 0.32	9.73 ± 0.39	5.23 ± 0.10	6.47 ± 0.19	7.17 ± 0.61	9.71 ± 0.43
Rhizoctonia solani	60	—	6.53 ± 0.13	6.87 ± 0.36	7.92 ± 0.44	—	5.17 ± 0.19	6.47 ± 0.40	7.60 ± 0.33
Valsa ceratosperma	24	7.57 ± 0.41	8.23 ± 0.14	9.00 ± 0.14	10.31 ± 0.19	7.19 ± 0.27	7.30 ± 0.31	7.89 ± 0.16	9.19 ± 0.43
Fusarium oxysporum f. sp. *Cucumis melo* L.	48	5.25 ± 0.38	6.39 ± 0.59	8.03 ± 0.19	9.26 ± 0.07	5.79 ± 0.26	6.27 ± 0.26	7.99 ± 0.27	9.55 ± 0.26
Fusarium graminearum	60	7.22 ± 0.24	8.25 ± 0.17	9.30 ± 0.25	11.03 ± 0.04	5.81 ± 0.14	7.72 ± 0.81	9.10 ± 0.16	10.69 ± 0.70
Bipolaris maydis	72	—	—	—	6.95 ± 0.08	—	—	—	8.51 ± 0.17
Colletotrichum orbiculare	60	9.11 ± 0.06	10.54 ± 0.57	11.20 ± 0.02	12.48 ± 0.46	9.88 ± 0.50	10.57 ± 0.46	11.07 ± 0.24	11.36 ± 0.92
Fusarium verticillioides[d]	72	—	6.59 ± 0.39	7.01 ± 0.20	7.93 ± 0.28	—	6.09 ± 0.67	6.90 ± 0.28	7.73 ± 0.22
Fusarium verticillioides[e]	60	—	6.23 ± 0.19	8.33 ± 0.47	9.55 ± 0.52	—	6.72 ± 0.30	7.32 ± 0.55	8.67 ± 0.20

[a]The data represented radius (mean ± s.d., mm) of antifungal zone.
[b]Each paper (diameter: 10 mm) contained 10 μl volume, the control only contained methanol (10 μl).
[c]The reaction time (h) between antibiotic and phytopathogens.
[d]An endophytic phytopathogen isolated from *Arctium lappa* L.
[e]An endophytic phytopathogen of *Gossypium* spp.

activity. Each concentration was conducted in triplicate and independently, and the diameters of inhibition zones were measured with a slide gauge. Then, the human leukemia (HL-60) cell line was used as a model to evaluate the cytotoxicity of lipopeptides based on the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay.

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