Two New Antifungal Cyclic Lipopeptides from *Bacillus marinus* B-9987

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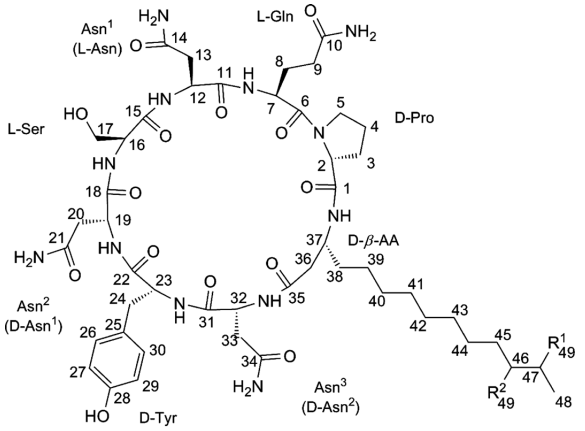
Two new cyclic lipopeptides maribasins A (1) and B (2) were isolated from the fermentation broth of the marine microorganism *Bacillus marinus* B-9987 isolated from *Suaeda salsa* in Bohai coastline of P.R. China. Both structures were established to be cyclo (D-Pro-L-Gln-L-Asn-L-Ser-D-Asn^1-D-Tyr-D-Asn^2-D-β-aminoisopentadecanoic acid) (1) and cyclo (D-Pro-L-Gln-L-Asn-L-Ser-D-Asn^1-D-Tyr-D-Asn^2-D-β-aminoanteisopentadecanoic acid) (2) by spectroscopic analysis and exhibited broad-spectrum activity against phytopathogens by the antifungal bioassay.

Key words *Bacillus marinus*; cyclic lipopeptide; antifungal activity; maribasin A; maribasin B

The number of new bioactive marine nature products has increased in recent years.[1,2] The strain *Bacillus marinus* B-9987, isolated from *Suaeda salsa* in Bohai coastline of P.R. China, has shown excellent activity against phytopathogens both *in vitro* and *in vivo*,[3,4] and some antimicrobial macrolactin constituents[5] have been isolated from this strain. In order to screen more constituents by bio-guided isolation, we have obtained two new cyclic lipopeptides maribasins A (**1**) and B (**2**) (Fig. 1) determined by chemical analysis and several different spectroscopic techniques. Cyclo lipopeptides were synthesized by a number of microorganisms including members of the *Bacillus* genus, cyanobacterium and some *Pseudomonas* species. Lipopeptides are classified into three families including surfactins, iturins and fengycins families according to their primary structure and their biological activity. The surfactin family encompasses structural variants, however, all members are heptapeptides interlinked with a β-hydroxy fatty acid to form a cyclic lactone ring structure. Iturin A and C, bacillomycin D, F, L and L_C, and mycosubtilin, seven main variants within the iturin family,[6] are heptapeptides linked to a β-amino fatty acid chain with a length of 14 to 17 carbons. The third family of fengycins includes fengycins A and B, which consists of a 10 amino acid peptide, in which residues 3—10 form a cyclic octapeptide, *via* a lactone bond for fengycins.[7] Several cyclo lipopeptides, cyclomarins A—C,[7] kurstakins,[8] mixirin A, B and C,[9] and scopularides A and B[10] have been discovered in recent years. These molecules exhibited biological activities such as antifungal, antitumor and antibacterial activity. Among them, mixirin A, B and C, members of the iturin class, exhibited antitumor activity *in vitro*.[9] This paper describes the isolation, structural elucidation and antifungal bioassay of the two new cyclic lipopeptides.



1: $R^1 = CH_3$, $R^2 = H$

2: $R^1 = H$, $R^2 = CH_3$

Fig. 1. Structures of Compounds **1** and **2**

Results and Discussion

Compound **1** was obtained as white amorphous powder by a multi-step chromatography procedure from the fermentation broth of the marine microorganism *B. marinus* B-9987. It gave an $[M+Na]^+$ peak in the high-resolution electrospray-ionization mass spectrometry (HR-ESI-MS) at *m/z* 1079.5505 (Calcd for $C_{49}H_{76}N_{12}O_{14}Na^+$, 1079.5502). The intense absorptions between 1600—1700 cm^{-1} and between 3100—3400 cm^{-1} in the IR spectrum suggested the presence of the amide C=O and NH groups, respectively. Compound **1** showed negative to ninhydrin but positive after hydrolysis with 6 M HCl, indicating that **1** contains cyclopeptide.[11]

The ^{1}H-NMR spectrum of **1** (Table 1) revealed the presence of 8 H-C(α) (δ_H 4.51, 4.45, 4.44, 4.43, 4.17, 4.16, 4.02, 4.01, partly overlapped, 1H each), 15 N-binding protons (δ_H 8.73, 8.73, 8.08, 7.73, 7.39, 7.34, 7.34, 7.23, 7.15, 7.14, 6.99, 6.93, 6.89, 6.87, 6.87, partly overlapped, 1H each), long methylene chain centered at δ_H 1.24 (16H, br s), 2 methyl groups (δ_H 0.84, 0.84, 3H each, t, J=6.6 Hz) and one para-substituted benzene ring (δ_H 7.03, 6.67, 2H each, d, J=8.1 Hz). The ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra of **1** (Table 1) exhibited 49 signals (Table 1), including 14 quaternary carbons in which 12 amide carbonyl carbons (δ_C 174.1, 173.3, 172.7, 171.8, 171.2, 171.2, 171.1, 171.0, 170.9, 170.8, 170.6, 170.3, partly overlapped) and 2 quaternary carbons of Tyr residue (δ_C 155.8, 127.9), 13 methine, 20 methylene and 2 methyl carbon. We could easily deduce that **1** was a lipopeptide composed of 1 Tyr, 3 Asn, 1 Pro, 1 Gln, 1 Ser residues and a β-amino acid with a–$(CH_2)_9CH(CH_3)_2$[12] group as a side chain by analysis of heteronuclear multiple quantum correlation (HMQC), heteronuclear multiple bond correlation (HMBC), ^{1}H–^{1}H correlation spectroscopy (COSY) and total correlation

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Table 1. ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) Data for Compounds **1** and **2** (DMSO-d_6)

Position	**1**		**2**	
	δ (H)	δ (C)	δ (H)	δ (C)
D-Pro				
1		173.3 (s)		173.3 (s)
2	4.16 (overlap)	60.8 (d)	4.16 (overlap)	60.8 (d)
3	2.14 (overlap), 1.78 (overlap)	29.0 (t)	2.14 (overlap), 1.77 (overlap)	28.6 (t)
4	2.02 (overlap), 1.89 (m)	24.6 (t)	2.02 (overlap), 1.89 (m)	24.6 (t)
5	3.77 (m)	47.2 (t)	3.77 (m)	47.2 (t)
N				
L-Gln				
6		170.9 (s)		170.9 (s)
7	4.51 (m)	49.6 (d)	4.51 (m)	49.6 (d)
8	2.02 (overlap), 1.78 (overlap)	26.5 (t)	2.02 (overlap), 1.77 (overlap)	26.5 (t)
9	2.12 (overlap)	30.6 (t)	2.12 (overlap)	30.6 (t)
10		174.1 (s)		174.1 (s)
NH_2	7.15 (overlap), 6.87 (overlap)		7.14 (overlap), 6.87 (overlap)	
NH	6.99 (d, 7.5)		6.99 (d, 7.5)	
Asn^{1} (L-Asn)				
11		171.0 (s)		171.0 (s)
12	4.43 (overlap)	49.6 (d)	4.43 (overlap)	49.6 (d)
13	2.71 (overlap), 2.50 (overlap)	35.2 (t)	2.71 (overlap), 2.50 (overlap)	35.2 (t)
14		171.8 (s)		171.8 (s)
NH_2	7.39 (s), 6.87 (overlap)		7.39 (s), 6.87 (overlap)	
NH	8.73 (overlap)		8.73 (overlap)	
L-Ser				
15		170.3 (s)		170.3 (s)
16	4.17 (overlap)	56.2 (d)	4.17 (overlap)	56.3 (d)
17	3.66 (s)	61.4 (t)	3.67 (s)	61.4 (t)
17-OH	4.89 (br s)		4.88 (br s)	
NH	7.34 (overlap)		7.35 (overlap)	
Asn^{2} (D-Asn^{1})				
18		170.8 (s)		170.8 (s)
19	4.45 (overlap)	50.8 (d)	4.45 (overlap)	50.8 (d)
20	2.58 (m), 2.50 (overlap)	36.0 (t)	2.58 (m), 2.49 (overlap)	36.0 (t)
21		171.2 (overlap)		171.2 (overlap)
NH_2	7.23 (s), 6.89 (s)		7.23 (s), 6.89 (s)	
NH	8.08 (d, 7.1)		8.08 (d, 7.1)	
D-Tyr				
22		171.1 (s)		171.2 (s)
23	4.01 (overlap)	56.3 (d)	4.02 (overlap)	56.2 (d)
24	2.97 (d, 11.3)	34.9 (t)	2.97 (d, 11.3)	34.9 (t)
	2.72 (overlap)		2.72 (overlap)	
25		127.9 (s)		127.9 (s)
26, 30	7.03 (d, 8.1)	129.7 (d)	7.03 (d, 8.1)	129.7 (d)
27, 29	6.66 (d, 8.1)	115.0 (d)	6.67 (d, 8.1)	115.1 (d)
28		155.8 (s)		155.8 (s)
28-OH	9.22 (s)		9.22 (s)	
NH	8.73 (overlap)		8.73 (overlap)	
Asn^{3} (D-Asn^{2})				
31		172.7 (s)		172.7 (s)
32	4.44 (overlap)	50.8 (d)	4.44 (overlap)	50.8 (d)
33	2.30 (m), 2.17 (overlap)	36.3 (t)	2.30 (m), 2.17 (overlap)	36.3 (t)
34		170.6 (s)		170.6 (s)
NH_2	7.34 (overlap), 6.93 (s)		7.35 (overlap), 6.94 (s)	
NH	7.73 (d, 3.1)		7.73 (d, 3.1)	
D-β-AA				
35		171.2 (overlap)		171.2 (overlap)
36	2.34 (m)	41.7 (t)	2.35 (m)	41.7 (t)
37	4.02 (overlap)	45.4 (d)	3.99 (overlap)	45.4 (d)
38	1.41 (s)	34.6 (t)	1.41 (s)	34.6 (t)
39	1.24 (br s), 1.15 (m)	28.6 (t)	1.24 (br s), 1.15 (m)	25.4 (t)
40—45	1.24 (br s)	29.2—25.4 (t)	1.24 (br s)	29.5—28.6 (t)
46	1.24 (br s)	38.5 (t)	1.24 (br s)	33.7 (d)
47	1.24 (br s)	27.4 (d)	1.24 (br s)	29.1 (t)
48	0.84 (t, 6.6)	22.5 (q)	0.82 d (t, 6.6)	11.2 (q)
49	0.84 (t, 6.6)	22.5 (q)	0.82 d (t, 6.6)	19.1 (q)
NH	7.14 (overlap)		7.15 (overlap)	

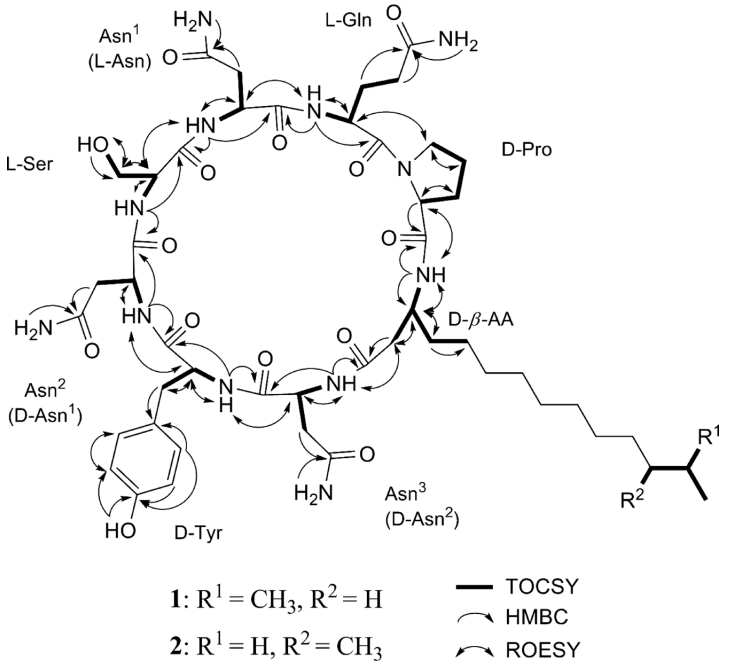

Fig. 2. Key Correlations of TOCSY, HMBC and ROESY of Compounds **1** and **2**

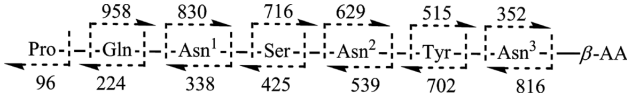

Fig. 3. MS^n Fragmentation of Compounds **1** and **2**

spectroscopy (TOCSY) spectra (Table 1, Fig. 2). These amino acid residues were also confirmed by amino acids analysis following hydrolysis of **1** at 110 °C. Key correlations between the NH (H-4 of Pro residue) and H-C(α) protons of neighbored residues in rotating frame Overhauser effect spectroscopy (ROESY) and NH and C=O of neighbored residues in HMBC (Fig. 2) finally allowed us to establish the structure of **1** as cyclo (Pro-Gln-Asn^1-Ser-Asn^2-Tyr-Asn^3-β-aminoisopentadecanoic acid). The sequence of the residues of **1** was also confirmed by multiple stages of collisionally-activated decomposition (CAD) in MS/MS (Fig. 3).

The absolute configuration of the amino acids of **1** was determined by the crude hydrolysate with Marfey's reagent[13] and HPLC analysis with co-injection of standards. The hydrolysate was identified to possess 1 D-Pro, 1 L-Glu, 1 L-Ser, 1 D-Tyr, 1 L-Asp and 2 D-Asp. There is no ambiguity in the four amino acids other than the Asn residues (in the form of the corresponding Asp). The absolute stereochemistries of the three Asn residues were proposed by cautious analysis of 2D ROESY spectra (Fig. 2). The ROESY correlation between α-proton (δ_H 4.51, m) of L-Gln and the amide proton (δ_H 6.99, d, J=7.5 Hz) of L-Gln, between the amide proton (δ_H 6.99, d, J=7.5 Hz) of L-Gln and the α-proton (δ_H 4.43, overlapped) of Asn^1 (L-Asn), between the α-proton (δ_H 4.43, overlapped) of Asn^1 (L-Asn) and the amide proton (δ_H 8.73, overlapped) of Asn^1 (L-Asn), and between the amide proton (δ_H 8.73, overlapped) of Asn^1 (L-Asn) and the α-proton (δ_H 4.17, overlapped) of L-Ser, revealed that the side chains of L-Pro, L-Gln, Asn^1 (L-Asn) and L-Ser, on the same side. It is easily suggested that Asn^2 and Asn^3 are both D-Asn residues by similar ROESY correlation. Thus, the structure of **1** was finally established to be cyclo (D-Pro-L-Gln-L-Asn-L-Ser-D-Asn^1-D-Tyr-D-Asn^2-D-β-aminoisopentadecanoic acid).

The main difference between **1** and **2** is the branching of the β-amino fatty acid chain (Table 1). ^{1}H-NMR, ^{13}C-NMR and HMBC spectra of **2** showed the presence of one terminal *sec*-butyl group, indicating that the side-chain of β-amino acid was $-(CH_2)_8CH(CH_3)CH_2CH_3$.[12]

In the present study, it was found that maribasins A and B are heptapeptides linked to a β-amino fatty acid chain with a length of 15 carbons. Further analysis showed the presence of one Tyr residue, one Ser, one Pro, one Gln and three Asn residues like iturin family. The major difference between maribasins and iturin family is the sequences of these seven amino acids. The sequence of maribasins with D-Pro-L-Gln-L-Asn-L-Ser-D-Asn-D-Tyr-D-Asn is different from that of iturins with L-Asn-D-Tyr-D-Asn-L-Gln-L-Pro-D-Asn-L-Ser. Most importantly, maribasins A and B possess four D-amino acids including one D-Tyr residue, one D-Pro and two D-Asn. However, iturins possess three D-amino acids including one D-Tyr residue and two D-Asn.

As shown in Table 2, both **1** and **2** exhibited broad-spectrum activities against phytopathogens such as *Alternaria solani*, *Fusarium oxysporum*, *Verticillium alboatrum*, *F. graminearum*, *Sclerotium* sp., *Penicillium* sp., *Rhizoctonia solani* and *Colletotrichum* sp. with the minimum inhibitory concentrations (MICs) at 25—200 μg/ml. In general, the antifungal activities of **1** and **2** are weaker than those of other cyclopeptides such as bacillomycin Fa (MIC for *B. cinerea* is 20 μg/ml)[14] iturin D (MICs for *F. oxysporum* and *Botrytis cinerea* are both 30 μg/ml)[15] and fengycin (MIC for *R. solani* is 3.16 μg/ml),[16] but their antifungal activity against *F. oxysporum* is stronger than that of bacillomycin Fa (MIC for *F. oxysporum* is >320 μg/ml).[14] In addition, it seems that the antifungal activities varied directly with the length of the side-chain of β-amino acid. Though the antifungal activity of

Table 2. Minimum Inhibitory Concentration Value for Plant Pathogens of Compounds **1** and **2**

Plant pathogens	Minimum inhibitory concentration value (μg/ml)	
	1	**2**
Alternaria solani	100	100
Fusarium oxysporum	200	>200
Verticillium alboatrum	100	200
Fusarium graminearum	100	200
Sclerotium sp.	25	100
Penicillium sp.	50	100
Botrytis cinerea	100	50
Drechslera turcica	100	100
Rhizoctonia solani	200	200
Colletotrichum sp.	50	50
Fusarium oxysporum f. sp. *cuberse*	100	100

1 against *B. cinerea* is weaker than that of **2**, the antifungal activity of **1** against *F. oxysporum*, *V. alboatrum*, *F. graminearum*, *Sclerotium* sp. and *Penicillium* sp. are stronger than those of **2**.

In this study, *B. marinus* strain B-9987, isolated from the rhizosphere soil of *Suaeda salsa* in Bohai coastline, was found to produce maribasins A and B as antifungal agents against many plant pathogens by the serial two-fold agar dilution method. The three families of *Bacillus* lipopeptides–surfactins, iturins and fengycins can influence the ecological fitness of the producing strain in terms of root colonization (and thereby persistence in the rhizosphere) and also play a role in the beneficial interaction of *Bacillus* species with plants by stimulating host defence mechanisms.[6] Therefore, these lipopeptides may play a role in the biocontrol of different plant pathogens. *B. marinus* strain B-9987 could possibly be used as a maribasins producer for regulating plant disease.

Experimental

General Experimental Procedure Fractions were monitored with TLC (HSGF 254, Yantai, P. R. China), and spots were visualized by heating silica gel plates sprayed with 5% H_2SO_4 in 95% ethanol. Column chromatography (CC) was performed on Sephadex LH-20 (Pharmacia) and silica gel (200—300 mesh, Yantai, P. R. China). HPLC purifications were carried out on a Waters 1525/2487 liquid chromatograph. UV spectra were performed on a Shimadzu-UV-1700 spectrophotometer. IR spectra were performed on a Magna-IR 550 spectrometer. NMR experiments were performed on a Bruker AVANCE-500 instrument. HR-ESI-MS spectrum was acquired using a Q-Tof micro LCTTM mass spectrometer. ESI-MS was performed on a Q-Tof micro instrument at a capillary 3 kV, a sample cone 80 V, an extraction cone 4 V, a source temperature 80 °C, a desolvation temperature 150 °C, an ion energy 1 V, MCP detector 2200 V and a collision energy 10 V (MS) or 40 V (MS/MS). Amino acids analysis was performed on a Hitachi L-8900 High speed amino acid analyzer. The melting point was measured on a SGW X-4 melting point apparatus and was uncorrected.

Bacterial Material The marine microorganism B-9987 was isolated from *S. salsa* in Bohai coastline, P. R. China. The strain was identified as *B. marinus* according to its morphological characters, biochemical characters and the partial sequence of its 16S ribosomal DNA (rDNA).[3] The strain was deposited both in Marine Pharmaceutical Bank of First Institute of Oceanography, State Oceanic Administration (HTTA-X99501), P. R. China and China General Microbiological Culture Collection Center (CGMCC No.2095).

Fermentation, Extraction, and Purification The strain B-9987 was incubated at 30 °C for 24 h in 50 l tank containing 30 l culture medium (Glucose 500.0 g; Sucrose 300.0 g; Yeast extract 400.0 g; albumen powder 400.0 g; $MgCl_2$ 10.0 g; KCl 5.0 g; KH_2PO_4 20.0 g; NaCl 100.0 g; pH 7.0). Thirty liters culture broth of B-9987 was centrifuged at 4000 rounds per minute for 10 min. The supernatant was then extracted three times with ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH), respectively. The EtOAc and *n*-BuOH extracts were then concentrated *in vacuo*. The *n*-BuOH extract was subjected to CC (silica gel) eluted with $CHCl_3$–MeOH system (20 : 1, 10 : 1, 6 : 1, 4 : 1, 2 : 1, 1 : 1, v/v), the active fraction of $CHCl_3$/MeOH 1 : 1 was then purified by CC (Sephadex LH-20) eluted with MeOH and finally purified by HPLC (Sunfire C18, 5 μm, 4.6×250 mm, 7 ml/min, UV detection 210 nm, t_R=36.2, 38.4 min) eluted with CH_3OH–H_2O (70 : 30, v/v) to yield maribasin B (**2**, 8 mg) and maribasin A (**1**, 15 mg) respectively.

Maribasin A (**1**), Cyclo (D-Pro-L-Gln-L-Asn-L-Ser-D-Asn^1-D-Tyr-D-Asn^2-D-β-Aminoisopentadecanoic Acid): White amorphous powder ($CHCl_3$/MeOH); mp >250 °C; $[\alpha]_D^{25}$ +0.26 (c=0.33, DMSO); UV λ_{max} (MeOH) nm (log ε): 210 (2.6). IR (KBr) cm^{-1}: 3333, 2929, 2864, 1659, 1545, 1449, 1423, 1247, 1128. HR-ESI-MS (positive-ionization mode) m/z: 1079.5505 $[M+Na]^+$, (Calcd for $C_{49}H_{76}N_{12}O_{14}Na^+$, 1079.5502); ^{1}H- and ^{13}C-NMR: see Table 1.

Maribasin B(**2**), Cyclo (D-Pro-L-Gln-L-Asn-L-Ser-D-Asn^1-D-Tyr-D-Asn^2-D-β-Aminoanteisopentadecanoic Acid): White amorphous powder ($CHCl_3$/MeOH); mp >250 °C; UV λ_{max} (MeOH) nm (log ε): 210 (2.6). HR-ESI-MS (positive-ionization mode) m/z: 1079.5507 $[M+Na]^+$, (Calcd for $C_{49}H_{76}N_{12}O_{14}Na^+$, 1079.5502); ^{1}H- and ^{13}C-NMR: see Table 1.

Amino Acid Analysis Total hydrolysis of **1** or **2** with 6 N HCl were carried out at 110 °C for 24 h. After the lipid parts were extracted from the hydrolysate, the aqueous phase was dried under vacuum. The amino acid analyzer (Hitachi L-8900, Japan) attached Hitachi HPLC Packed Column with Ion-exchanging Resin No. 2622 PF (4.6×60 mm) and UV detector (570, 440 nm) was used for analysis of amino acids. 20 μl of each sample was injected and determination was performed using Ninhydrin reagent set (Wako Chemical Inc., Japan).

Absolute Stereochemistry of Amino Acid Analysis by Marfey's Derivatization Maribasins A (**1**, 1.8 mg) and B (**2**, 1.8 mg) were hydrolyzed with 6 N HCl , dried *in vacuo*. The hydrolyzes were added H_2O (35 μl), 1% FDAA (Marfey's reagent, 420 μl) in acetone, and 1 M $NaHCO_3$ (84 μl). The mixture was heated at 40 °C for 1 h, quenched by addition of 2 M HCl (7 μl), and evaporated to dryness to furnish a yellow solid. The residue was dissolved in H_2O (500 μl) and analyzed by reversed-phase HPLC (Sunfire C18, 4.6×150 mm, UV detection at 340 nm, flow rate 1 ml/min) using a linear gradient (10% CH_3CN in H_2O containing 0.05% TFA to 50% CH_3CN within 60 min). Co-injection with standard L- and D-amino acids confirmed that both hydrolyzates contained D-Pro, L-Glu, L-Ser, D-Tyr, L-Asp and D-Asp with a 1 : 2 molar ratio of L-Asp to D-Asp.

Bioactive Tests The antifungal activities of **1** and **2** were determined by the serial two-fold agar dilution method using potato dextrose agar media for plant pathogens including *Alternaria solani*, *Fusarium oxysporum*, *Verticillium*, *F. graminearum*, *Sclerotium* sp., *Penicillium* sp., *B. cinerea*, *Drechslera turcica*, *Rhizoctonia solani*, *Colletotrichum* sp. and *F.* f. sp. *cuberse* after incubation for 48 h at 25 °C. Both of **1** and **2** were prepared as stock solutions and serial twofold dilutions were performed. The final concentrations ranged from 6.25 to 200 μg/ml for **1** and **2**. The inoculum suspensions of plant pathogens were prepared from 7-d cultures grown on potato dextrose agar at 25 °C. Each suspension was inoculated into potato dextrose agar media to obtain the final inoculum concentration of 0.4 to 5×10^4 cells/ml. Steel perforator was inserted into these plates after the medium had solidified, in order to produce holes of 10.0 mm diameter. The holes were then filled with 60 μl of specific **1** and **2** concentrations. The plates were incubated for 48 h at 25 °C. For all the antifungal agents tested, the MIC was read as the lowest drug concentration that prevented any discernible growth.

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References

1) Blunt J. W., Copp B. R., Munro M. H. G., Northcote P. T., Prinsep M. R., *Nat. Prod. Rep.*, **27**, 165—237 (2010).
2) Mayer A. M. S., Rodríguez A. D., Berlinck R. G. S., Hamann M. T., *Biochim. Biophys. Acta*, **1790**, 283—308 (2009).
3) Luo Y. C., Tian L., Han F. F., Li Y. G., *Agrochemicals*, **47**, 691—693 (2008).
4) Tian Li, Gu Z. F., Chen J., Huang L. P., Tian Ling, *Acta Phytopatho-*

logica Sinica, **33**, 77—80 (2003).
5) Xue C. M., Tian L., Xu M. J., Deng Z. W., Lin W. H., *J. Antibiot.*, **61**, 668—674 (2008).
6) Ongena M., Jacques P., *Trends Microbiol.*, **16**, 115—125 (2008).
7) Renner M. K., Shen Y. C., Cheng X. C., Jensen P. R., Frankmoelle W., Kauffman C. A., Fenical W., Lobkovsky E., Clardy., *J. Am. Chem. Soc.*, **121**, 11273—11276 (1999).
8) Hathout Y., Ho Y. P., Ryzhov V., Demirev P., Fenselau C., *J. Nat. Prod.*, **63**, 1492—1496 (2000).
9) Zhang H. L., Hua H. M., Pei Y. H., Yao X. S., *Chem. Pharm. Bull.*, **52**, 1029—1030 (2004).
10) Yu Z. G., Lang G., Kajahn I., Schmaljohann R., Imhoff J. F., *J. Nat. Prod.*, **71**, 1052—1054 (2008).
11) Zhou J., Tan N. H., *Chin. Sci. Bull.*, **45**, 1825—1831 (2000).
12) Hiradate S., Yoshida S., Sugie H., Yada H., Fujii Y., *Phytochemistry*, **61**, 693—698 (2002).
13) Marfey P., *Carlsberg Res. Commun.*, **49**, 591—596 (1984).
14) Moyne A. L., Cleveland T. E., Tuzun S., U.S. Patent 6183736B1 (2001).
15) Besson F., Michel G., *J. Antibiot.*, **40**, 437—442 (1987).
16) Vanittanakom N., Loeffler W., Koch U., Jung G., *J. Antibiot.*, **39**, 888—901 (1986).