JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Production of Bacillomycin- and Macrolactin-Type Antibiotics by *Bacillus amyloliquefaciens* NJN-6 for Suppressing Soilborne Plant Pathogens

Jun Yuan, Bing Li, Nan Zhang, Raza Waseem, Qirong Shen,[†] and Qiwei Huang*[,†]

Jiangsu Key Laboratory for Organic Solid Waste Utilization, Nanjing Agricultural University, Nanjing 210095, People's Republic of China

ABSTRACT: *Bacillus amyloliquefaciens* strains have been used as biocontrol agents for the suppression of several soilborne plant pathogens. A clearer understanding of the antagonistic mechanisms of action of these bacteria will facilitate their use in the control of plant diseases. Antagonistic substances were isolated from the fermentation broth of *B. amyloliquefaciens* strain NJN-6 cultures. These compounds were preconcentrated using an XAD-16 column and were purified using reversed-phase high-performance liquid chromatography (RP-HPLC). Fractions were collected from the column and were analyzed, and two homologues of bacillomycin D [molecular weights of 1030 Da (C14) and 1044 Da (C15)] and three homologues of members of the macrolactin family, macrolactin A, 7-*O*-malonyl macrolactin A, and 7-*O*-succinyl macrolactin A (molecular weights of 402, 487, and 502 Da, respectively) were identified using HPLC/electrospray ionization mass spectrometry (ESI−MS) analysis. An antagonistic assay showed that bacillomycin D and macrolactin exhibited significant antagonistic effects against *Fusarium oxysporum* and *Ralstonia solanacearum*, respectively. A reliable method for the isolation and purification of bacillomycin D and macrolactin from bacterial broth cultures was developed. These data will help elucidate the mechanisms that *B. amyloliquefaciens* NJN-6 uses for the biocontrol of soilborne plant pathogens.

KEYWORDS: *Bacillus amyloliquefaciens, bacillomycin D, macrolactin, HPLC/ESI−MS analysis, biocontrol*

INTRODUCTION

Bacillus species are used as biocontrol agents for the suppression of many soilborne plant pathogens, such as *Rhizoctonia solani*, *Sclerotium rolfsii*,[1] *Sclerotinia sclerotiorum*,[2] and *Fusarium oxysporum*.[3] The biocontrol activities of *Bacillus* sp. involve a number of mechanisms, such as competition, antagonism, induction of systemic resistance, and promotion of plant growth. Indeed, the production of antibiotics plays a major role in disease suppression.[4,5] A large number of *Bacillus* strains have been screened over the last several decades. These strains are the potential producers of numerous antibiotics, predominantly lipopeptides, such as surfactins, fengycins, and iturins.[6] All lipopeptides are amphiphilic molecules that vary in their peptide or fatty acid moieties. *Bacillus* sp. can also produce polyketides, a large family of secondary metabolites, including macrolactin, difficidin, and oxidifficidin.[7] These molecules have been used as antiseptics in agricultural and medical applications.

Iturins are lipopeptides that are synthesized by non-ribosomal peptide synthetases and consist of many families. Bacillomycin, a member of the iturin family of peptides, contains one β-amino fatty acid and seven α-amino acids in its molecular structure. Bacillomycin homologues are referred to as bacillomycins D, F, and L, and they differ in the composition of their amino acids and length of their fatty acid chain, which varies from C14 to C17. Similar to other iturin homologues, these compounds exhibit strong antifungal activities and limited antibacterial activities.[8,9] In comparison to lipopeptides, which were first isolated from a deep-sea bacterium, macrolactins are relatively new antibiotics.[10] More than 17 macrolactins have been characterized, such as macrolactin A,[11] F,[12] N,[13] S,[14] and W.[15] Macrolactin A exhibits efficient antibacterial activity, in that it inhibits cell division in *Staphylococcus aureus*.[11]

The *Bacillus amyloliquefaciens* strain NJN-6 used was isolated in our laboratory from the rhizosphere of a healthy banana plant, and it has an antagonistic effect on *F. oxysporum* f. sp. *cubense in vitro*. When this *Bacillus* strain was fermented with an organic fertilizer to generate a bio-organic fertilizer, it demonstrated excellent biocontrol activity in pot and in field experiments. This bio-organic fertilizer significantly decreased the incidence of *Fusarium* wilt of banana compared to that of the control. In a previous study, iturin A was isolated and quantified from the fermentation broth of *B. amyloliquefaciens* strain NJN-6.[16] In addition to the antifungal iturin A substance, other antimicrobial compounds may also be present in the broth. This study was carried out to purify and identify the other antimicrobial compounds that would inhibit some soilborne pathogens to provide theoretic support for the biological control application.

MATERIALS AND METHODS

Microorganisms. The Key Laboratory of Solid Organic Waste Utilization, Nanjing Agricultural University, Nanjing, China, isolated the antagonistic strain NJN-6 (CGMCC accession number 3183, China General Microbiology Culture Collection Center) from a soil sample and identified it as *B. amyloliquefaciens* using 16S rRNA sequencing. The 16S rRNA gene sequences of NJN-6, which showed

Received: November 28, 2011
Revised: February 29, 2012
Accepted: March 4, 2012
Published: March 4, 2012

100% identity to *B. amyloliquefaciens* strain CAU-B946 (accession number HE617159), was deposited into GenBank under accession number GQ452909. The strain was grown on Luria−Bertani (LB) agar plates and was recultured after 1 month.

Culture Medium and Growth Conditions. The *B. amyloliquefaciens* strain NJN-6 was incubated in LB medium (10 g of tryptone, 5 g of yeast extract, and 10 g of NaCl per liter). For the cultivation of pathogenic bacteria, solid NA medium (10 g of glucose, 5 g of tryptone, 0.5 g of yeast extract, 3 g of beef extract, and 30 g of agar per liter at pH 7) was used, and potato dextrose agar medium (200 g of potato infusion, 20 g of dextrose, 20 g of agar in 1 L of distilled water at pH 5.6 ± 0.2) was used to grow the pathogenic fungal strain.

Partial Purification of the Antagonistic Substances from *B. amyloliquefaciens* NJN-6. For the production of antagonistic substances, the fermentation of the NJN-6 strain was performed in 500 mL Erlenmeyer flasks with a 100 mL working volume. The LB cultures were incubated in a rotary incubator at 37 °C and 170 rpm for 56 h. The culture medium was centrifuged at 12000*g* (4 °C) for 10 min, and the supernatant was collected.

To prepare the samples for further purification by high-performance liquid chromatography (HPLC) and antagonistic activity experiments, 50 mL of cell-free cultures of the NJN-6 strain was passed through an Amberlite XAD-16 (Alfa Aesar, a Johnson Matthey Company, Ward Hill, MA) column (10 g). The antibiotics and impurities were bound to the column matrix. To remove the impurities, a gradient of different concentrations of methanol ranging from 0 to 100% (at increments of 10%) was used, and an antagonistic assay of collected elutes showed that 40% aqueous methanol could remove the impurities, with active compounds remaining in the column. Therefore, first, the column was washed with 200 mL of 40% methanol, and then elution of active compounds was performed using 20 mL of 100% methanol. The eluates were dried in a rotation evaporator (<40 °C) and dissolved in 2 mL of methanol.

Purification by HPLC. HPLC was performed using a HPLC 1200 device (1200 series, Agilent, Santa Clara, CA) to purify the active substances. A 20 μL sample, pretreated using an XAD-16 column, was injected into the HPLC column (Eclipse XDB-C18, 4.6 × 250 mm, 5 μm, Agilent, Santa Clara, CA). The temperature of the column was maintained at 20 °C throughout the experiment. The purification was performed using a solvent containing 60% A [0.1% (v/v) CH_3COOH] and 40% B (CH_3CN) at a flow rate of 0.6 mL/min. To produce a stable baseline, the column was equilibrated using the 60% A and 40% B solvent. An ultraviolet (UV) detector was used to detect peaks at 230 nm.

A fraction collector (Analyt FC, G1364C, Agilent, Santa Clara, CA) was used to collect the pure compounds, and the fractions were collected using the time and peaks mode. The injections were performed repeatedly to allow for the collection of a sufficient quantity of antibiotics. The fractions were lyophilized, and the residues were dissolved in 500 μL of methanol for mass spectrometry (MS) analysis and antagonistic activity assays.

MS Analysis. A total of five samples were collected from the HPLC fraction collector, and the molecular weight and molecular formula of each antifungal substance was determined using a liquid chromatography/electrospray ionization−mass spectrometry (LC/ESI−MS) system (1200 series, Agilent, Santa Clara, CA, and ESI−MS, 6410 Triple Quad LC/MS, Agilent, Santa Clara, CA) with a C18 column (50 × 2.1 mm, 1.8 μm) at a flow rate of 0.4 mL/min. The mobile phase was the same as the Purification by HPLC section. For MS analysis, the electrospray needle was operated at a spray voltage of 4.5 kV. The capillary temperature was 300 °C. Every sample was measured in the negative- and positive-ion modes for the assessment of accurate molecular weights for the antibiotics, and the mass spectra were acquired in an m/z range of 50−1200 at a scan rate of 500 atomic mass units (amu)/s.

Assay of Antagonistic Activity. The ability of antibiotics from *B. amyloliquefaciens* strain NJN-6 to inhibit the growth of the soilborne pathogenic fungus *F. oxysporum* f. sp. *cubense* (race 4) *in vitro* was determined in a solid medium growth assay. An agar plug of growing *F. oxysporum* (0.5 cm in diameter) was placed in the center of a potato dextrose agar (PDA) plate, and the HPLC-purified active compounds (50 μL) were placed into each of three wells surrounding the plug. The PDA plate was incubated at 28 °C for 4 days. Methanol was used as a negative control.

The antibacterial activity of active compounds against the soilborne pathogenic bacterium *Ralstonia solanacearum* (race 1, biovar 3) was tested using a NA plate. Top agar containing 100 μL of the bacterial suspension [10^8 colony forming units (CFU)/mL] was poured onto the plate. The HPLC-purified antagonistic substances (50 μL) were placed into wells in the top agar. The NA plate was incubated at 28 °C for 3 days. Methanol was used as a negative control.

RESULTS AND DISCUSSION

Partial Purification of the Antagonistic Substances from Cell-Free Supernatants. The XAD-16 resin is ideal for the partial purification of antibiotics because of its physical properties, which include porosity, uniform pore size distribution, high surface area, non-ionic structure, and ability to adsorb large amounts of uncharged compounds.[17] Chen et al.[18] purified polyketides using XAD-16. In our experiment, as many impurities as possible were removed by washing with different concentrations of aqueous methanol. The results suggested (Figure 1) that washing with 40% methanol solution

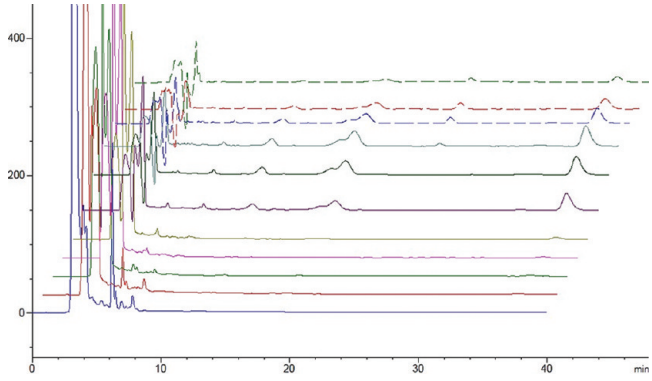

Figure 1. Effect of different concentrations of methanol on the purification of active compounds. A series of different concentrations of methanol ranging from 0 to 100% at increments of 10% were evaluated.

excluded most of the impurities, and the purified antibiotics were eluted using 100% methanol. Using this methodology, the antibiotics from 50 mL of cell-free liquid culture were partially purified and concentrated to 2 mL.

HPLC Purification and MS Analysis of the Antagonistic Substances. The partially purified compounds were subjected to reversed-phase (RP)-HPLC for purification. Peaks (five in total) with retention times of 11.93, 17.97, 24.89, 33.82, and 34.99 min were collected using a fraction collector and were recorded as 1, 2, 3, 4, and 5, respectively. The collection process is shown in Figure 2. The elutes were lyophilized and dissolved in methanol.

To determine the molecular weight and molecular formula of the five HPLC-purified antibiotics, the samples were subjected to HPLC analysis coupled with ESI−Q-trap MS. The data from these HPLC/ESI−MS analyses are shown in Figures 3 and 4. The spectra of 1 revealed molecular masses of 1029.1 Da $[M - H]^-$ in the negative-ion mode (Figure 3A) and 1031.5 Da $[M + H]^+$ and 1053.5 Da $[M + Na]^+$ in the positive-ion mode (Figure 3B). Therefore, 1 was identified as bacillomycin D with a molecular weight of 1030 Da (C14) compared to published studies.[19,20] The spectra of 2 exhibited a molecular mass of

dx.doi.org/10.1021/jf204868z | J. Agric. Food Chem. 2012, 60, 2976−2981

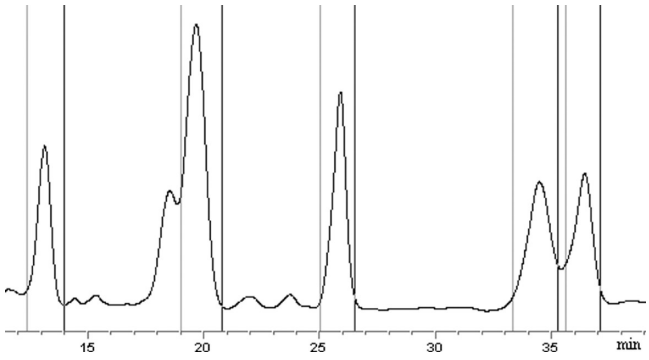

Figure 2. Eluates containing active compounds were obtained using the HPLC fraction collector.

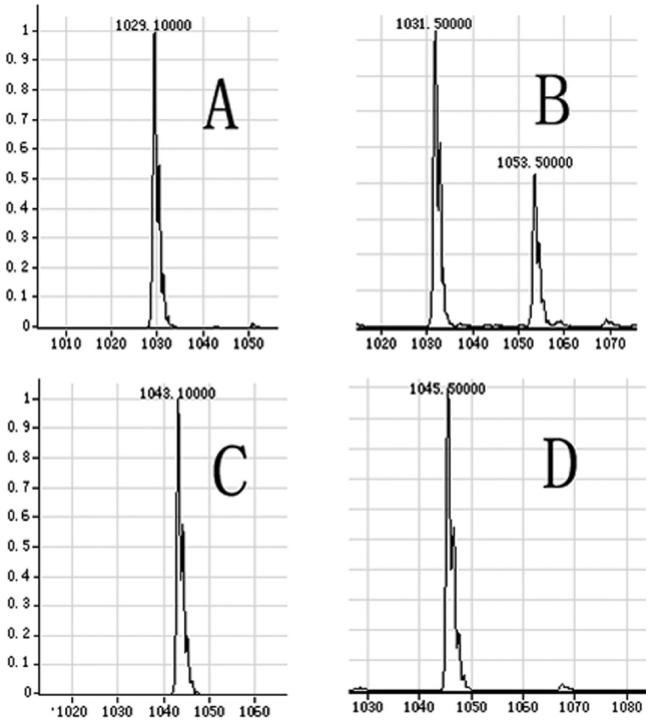

Figure 3. ESI mass spectra of bacillomycin D produced by *B. amyloliquefaciens* NJN-6: (A) mass spectra of bacillomycin D (C14) detected in negative-ion mode, (B) mass spectra of bacillomycin D (C14) detected in positive-ion mode, (C) mass spectra of bacillomycin D (C15) detected in negative-ion mode, and (D) mass spectra of bacillomycin D (C15) detected in positive-ion mode.

1043.1 Da $[M - H]^-$ in the negative-ion mode and 1045.5 Da $[M + H]^+$ in the positive-ion mode. Therefore, 2 was identified as bacillomycin D with a molecular weight of 1044 Da (C15) (panels C and D of Figure 3).[19,20] The mass spectra of 3, 4, and 5 exhibited a m/z ratio of 401, 487, and 501.1 $[M - H]^-$, respectively, and $[M + Na]^+$ and $[M + K]^+$ ions were also found under positive-ion mode conditions. In addition, other prominent ions were present that helped determine the molecular weights of these compounds. The three compounds exhibited molecular weights of 402, 488, and 502 Da and were identified as macrolactin A, 7-*O*-malonyl macrolactin A, and 7-*O*-succinyl macrolactin A, respectively. The prominent masses from the HPLC/ESI−MS spectra and the molecular weights are listed in Table 1.

Assay of Antagonistic Activity. In Figure 5, the white on the right was the mycelium of *F. oxysporum* f. sp. *cubense* and

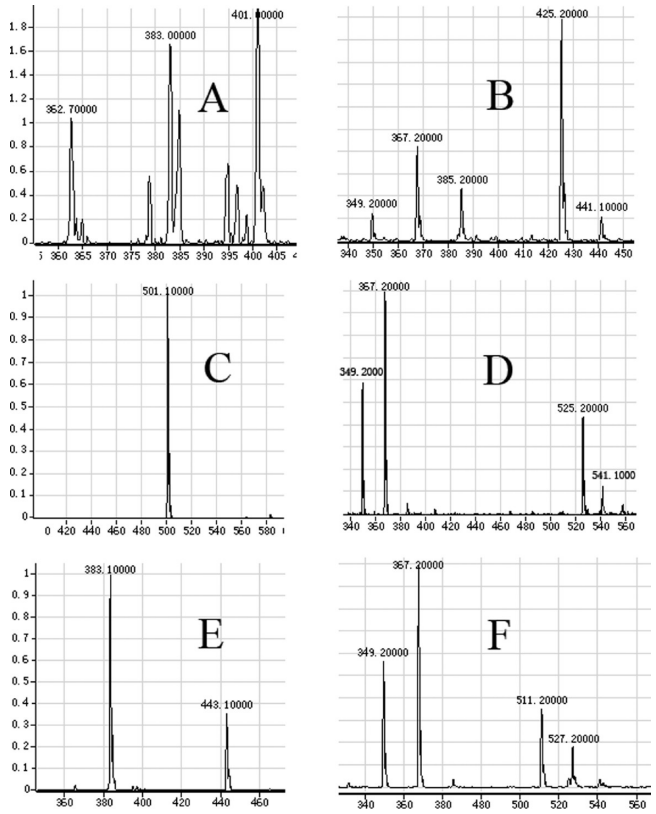

Figure 4. ESI mass spectra of macrolactin produced by *B. amyloliquefaciens* NJN-6: (A) mass spectra of macrolactin A detected in negative-ion mode, (B) mass spectra of macrolactin A detected in positive-ion mode, (C) mass spectra of 7-*O*-malonyl macrolactin A detected in negative-ion mode, (D) mass spectra of 7-*O*-malonyl macrolactin A detected in positive-ion mode, (E) mass spectra of 7-*O*-succinyl macrolactin A detected in negative-ion mode, and (F) mass spectra of 7-*O*-succinyl macrolactin A detected in positive-ion mode.

active compounds were added into the well of panels A and B, while methanol was added into the well of panel C as a control. In comparison to panel C, the growth of mycelium in panels A and B was inhibited by active compounds, and the diameters of the inhibition ring formed around the wells in panels A and B were 0.6 and 0.7 cm, respectively. Purified bacillomycin D from *B. amyloliquefaciens* strain NJN-6 demonstrated antifungal activity against *F. oxysporum*. However, no obvious antibacterial activity was observed in the antibacterial assay. The toxicity of the three macrolactins toward soilborne plant pathogens was studied. In Figure 6, the white points were the lawns of *R. solanacearum* and active compounds were added into the well of panels B−D, while methanol was added into the well of panel A as a control. In comparison to panel A, the growth of bacteria in panels B−D was inhibited by active compounds, and the diameters of the inhibition ring formed around the wells in panels B, C, and D were 1.2, 1.0, and 1.3 cm, respectively. The results showed that the compounds demonstrated an efficient antagonistic activity against the Gram-negative pathogenic bacteria *R. solanacearum*. In addition, these macrolactins exhibited antifungal activity against *F. oxysporum*; however, this antifungal activity was less than that demonstrated by bacillomycin D in the antifungal assay.

Soilborne plant diseases are a worldwide problem that decreases the quantity and quality of crops and fruits, especially under continuous-crop conditions. Biocontrol agents are

Table 1. Macrolactin Production by *B. amyloliquefaciens* NJN-6 Detected by HPLC/ESI−MS

		product ions		*m/z*			
number	retention time	negative	positive	negative	positive	molecular weight	compound
1	11.938	$[M - H]^-$	$[M + H]^+$	1029.1	1031.5	1030	bacillomycin D (C14)
			$[M + Na]^+$		1053.5		
2	17.970	$[M - H]^-$	$[M + H]^+$	1043.1	1045.5	1044	bacillomycin D (C15)
3	24.896	$[M - H]^-$	$[M + K]^+$	401	441.1	402	macrolactin A
		$[M - H_2O - H]^-$	$[M + Na]^+$	383	425.2		
			$[M - H_2O + H]^+$		385.2		
			$[M - 2H_2O + H]^+$		267.2		
			$[M - 3H_2O + H]^+$		349.2		
4	33.829	$[M - H]^-$	$[M + K]^+$	487	527.2	488	7-*O*-malonyl macrolactin A
		$[M - H - CO_2]^-$	$[M + Na]^+$	443.1	511.2		
		$[M - H$−malonic acid$]^-$	$[M$−malonic acid$-2H_2O + H]^+$	383	367.2		
			$[M$−malonic acid$-3H_2O + H]^+$		349.2		
5	34.994	$[M - H]^-$	$[M + K]^+$	501.1	541.1	502	7-*O*-succinyl macrolactin A
			$[M + Na]^+$		525.2		
			$[M$−succinic acid$-2H_2O + H]^+$		367.2		
			$[M$−succinic acid$-3H_2O + H]^+$		349.2		

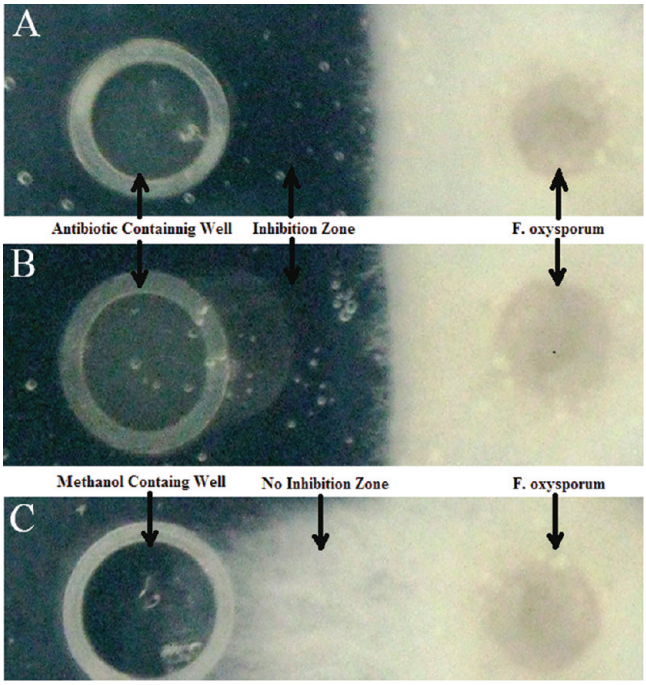

Figure 5. Antifungal activity of bacillomycin D against *F. oxysporum*: antifungal activity of (A) bacillomycin D (C14), (B) bacillomycin D (C15), and (C) methanol. The PDA plate was incubated at 28 °C for 4 days.

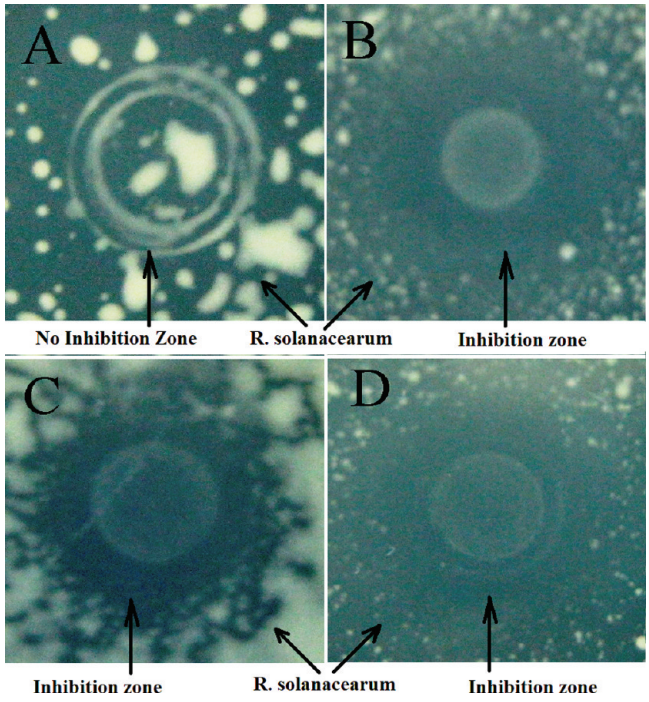

Figure 6. Antifungal activity of macrolactin against *R. solanacearum*: (A) control and antibacterial activity of (B) macrolactin A, (C) 7-*O*-malonyl macrolactin A, and (D) 7-*O*-succinyl macrolactin A. The plate was incubated at 28 °C for 3 days.

thought to decrease the incidence of these diseases. *Bacillus* is the most widely used biocontrol agent, and it produces a large number of antibiotics. Lipopeptides produced by *Bacillus* are regarded as versatile weapons against plant disease. These compounds have been studied for their antagonistic activity against a wide range of potential phytopathogens, and they play an important role in the beneficial interaction between *Bacillus* species and plants by stimulating host defense mechanisms.[21] Bacillomycin D, a type of iturin, is a heptapeptide that is linked to a β-amino fatty acid chain with a length of 14−17 carbons. *In vitro*, this compound demonstrates a strong antifungal activity against a wide variety of yeast and fungi but exhibits limited antibacterial activity.[21] In our study, bacillomycin D exhibited an adequate antagonistic effect on *F. oxysporum*; however, a limited antagonistic effect on *R. solanacearum* was observed (Figure 5). The results from this study are in agreement with other published studies.[9]

Macrolactin A was first isolated from a deep-sea bacterium, and it was used in clinical antibiosis because of its selective antibacterial activities, its cytotoxicity against B16-F-10 murine melanoma cancer cells, and its antiviral activities against Herpes simplex virus (HSV) and human immunodeficiency virus (HIV).[22] 7-*O*-Malonyl macrolactin A (MMA) is a bacteriostatic antibiotic that inhibits a number of multidrug-resistant Gram-positive bacteria pathogens. It inhibits one or more of the stages of cell division, and it interferes with the synthesis of the cell

dx.doi.org/10.1021/jf204868z | *J. Agric. Food Chem.* 2012, 60, 2976−2981

wall.[11] 7-*O*-Succinyl macrolactin A has the same configuration as its parent molecule macrolactin A, and it has an antagonistic effect on Gram-positive bacteria, such as *Bacillus subtilis* and *S. aureus*.[12] We used the Gram-negative pathogenic bacteria *R. solanacearum* as an indicator strain and demonstrated that macrolactin exerts an efficient antagonistic effect on this pathogen. The results also explain how *B. amyloliquefaciens* strain NJN-6, which produces macrolactin, is successful in controlling plant pathogenic bacteria. In our experiment, macrolactin demonstrated minimal antifungal activity against *F. oxysporum* as an indicator strain; however, another polyketide (difficidin) has demonstrated antifungal activity against *Erwinia amylovora* and is used to control fire blight disease.[18] Moreover, in the antibacterial activity assay, a number of *R. solanacearum* colonies appeared in the inhibition zone as the incubation time increased, and the inhibition zone became smaller and smaller. Thus, macrolactin seemed to restrict the growth of bacteria (bacteriostatic) rather than killing it (bactericidal). A similar phenomenon was observed in the antifungal activity experiment of bacillomycin D, which is an antifungal agent but did not kill fungi. Bacillomycin D is a type of iturin that is thermally stable even at 121 °C for 20 min.[23] In our experiments, macrolactin was maintained at room temperature for 1 month before detection by HPLC. The retention time of macrolactin was the same before and after this incubation period, and its peak areas did not decrease significantly. These results demonstrate that macrolactin is a stable compound. Macrolactin-type antibiotics were isolated from a deep-sea bacterium originally almost 20 years ago, and bacillomycin-type antibiotics were found much earlier than macrolactin. From the antibiotics isolated, no novel findings were supplied in our research. However, the antibiotics producer *B. amyloliquefaciens* NJN-6 was isolated from healthy banana rhizosphere by our laboratory and successfully used for the control of *Fusarium* wilt of banana. Furthermore, methodology in our paper is clearly described and correctly applied, which will used for the isolation and identification of other active compounds.

AUTHOR INFORMATION

Corresponding Author

*Telephone: 0086-25-84396212. E-mail: qwhuang@njau.edu.cn.

Author Contributions

†These authors contributed equally to this work.

Funding

This research was financially supported by the Agricultural Ministry of China (201103004) and the Nature Science Foundation of China (31070462).

Notes

The authors declare no competing financial interest.

REFERENCES

(1) De Curtis, F.; Lima, G.; Vitullo, D.; De Cicco, V. Biocontrol of *Rhizoctonia solani* and *Sclerotium rolfsii* on tomato by delivering antagonistic bacteria through a drip irrigation system. *Crop Prot.* **2010**, *29*, 663−670.

(2) Fernando, W. G. D.; Nakkeeran, S.; Zhang, Y.; Savchuk, S. Biological control of *Sclerotinia sclerotiorum* (Lib.) de Bary by *Pseudomonas* and *Bacillus* species on canola petals. *Crop Prot.* **2007**, *26*, 100−107.

(3) Zhang, N.; Wu, K.; He, X.; Li, S.-q.; Zhang, Z.-h.; Shen, B.; Yang, X.-m.; Zhang, R.-f.; Huang, Q.-w.; Shen, Q.-r. A new bioorganic fertilizer can effectively control banana wilt by strong colonization with *Bacillus subtilis* N11. *Plant Soil* **2011**, 1−11.

(4) Cook, R. J.; Thomashow, L. S.; Weller, D. M.; Fujimoto, D.; Mazzola, M.; Bangera, G.; Kim, D.-S. Molecular mechanisms of defense by rhizobacteria against root disease. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 4197−4201.

(5) Klich, M. A.; Arthur, K. S.; Lax, A. R.; Bland, J. M. Iturin A: A potential new fungicide for stored grains. *Mycopathologia* **1994**, *127*, 123−127.

(6) Raaijmakers, J. M.; de Bruijn, I.; Nybroe, O.; Ongena, M. Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: More than surfactants and antibiotics. *FEMS Microbiol. Rev.* **2010**, *34*, 1037−1062.

(7) Chen, X. H.; Koumoutsi, A.; Scholz, R.; Schneider, K.; Vater, J.; Sussmuth, R.; Piel, J.; Borriss, R. Genome analysis of *Bacillus amyloliquefaciens* FZB42 reveals its potential for biocontrol of plant pathogens. *J. Biotechnol.* **2009**, *140*, 27−37.

(8) Koumoutsi, A.; Chen, X.-h.; Henne, A.; Liesegang, H.; Hitzeroth, G.; Franke, P.; Vater, J.; Borriss, R. Structural and functional characterization of gene clusters directing nonribosomal synthesis of bioactive cyclic lipopeptides in *Bacillus amyloliquefaciens* strain FZB42. *J. Bacteriol.* **2004**, *186*, 1084−1096.

(9) Thimon, L.; Peypoux, F.; Wallach, J.; Michel, G. Effect of the lipopeptide antibiotic, iturin A, on morphology and memberane ultrastructure of yeast cells. *FEMS Microbiol. Lett.* **1995**, *128*, 101−106.

(10) Gustafson, K.; Roman, M.; Fenical, W. The macrolactins, a novel class of antiviral and cytotoxic macrolides from a deep-sea marine bacterium. *J. Am. Chem. Soc.* **1989**, *111*, 7519−7524.

(11) Romero-Tabarez, M.; Jansen, R.; Sylla, M.; Lunsdorf, H.; Haussler, S.; Santosa, D. A.; Timrnis, K. N.; Molinari, G. 7-*O*-Malonyl macrolactin A, a new macrolactin antibiotic from *Bacillus subtilis* active against methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, and a small-colony variant of *Burkholderia cepacia*. *Antimicrob. Agents Chemother.* **2006**, *50*, 1701−1709.

(12) Jaruchoktaweechai, C.; Suwanborirux, K.; Tanasupawatt, S.; Kittakoop, P.; Menasveta, P. New macrolactins from a marine *Bacillus* sp. Sc026. *J. Nat. Prod.* **2000**, *63*, 984−986.

(13) Yoo, J. S.; Zheng, C. J.; Lee, S.; Kwak, J. H.; Kim, W. G. Macrolactin N, a new peptide deformylase inhibitor produced by *Bacillus subtilis*. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4889−4892.

(14) Sohn, M. J.; Zheng, C. J.; Kim, W. G. Macrolactin S, a new antibacterial agent with FabG-inhibitory activity from *Bacillus* sp. AT28. *J. Antibiot.* **2008**, *61*, 687−691.

(15) Mondol, M. A. M.; Kim, J. H.; Lee, H.-S.; Lee, Y.-J.; Shin, H. J. Macrolactin W, a new antibacterial macrolide from a marine *Bacillus* sp. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3832−3835.

(16) Yuan, J.; Raza, W.; Huang, Q.; Shen, Q. Quantification of the antifungal lipopeptide iturin A by high performance liquid chromatography coupled with aqueous two-phase extraction. *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.* **2011**, *879*, 2746−2750.

(17) Wuilloud, R. G.; Acevedo, H. A.; Vazquez, F. A.; Martinez, L. D. Determination of lead in drinking water by ICP−AES with ultrasonic nebulization and flow-injection on-line preconcentration using an Amberlite XAD-16 resin. *Anal. Lett.* **2002**, *35*, 1649−1665.

(18) Chen, X. H.; Scholz, R.; Borriss, M.; Junge, H.; Mogel, G.; Kunz, S.; Borriss, R. Difficidin and bacilysin produced by plant-associated *Bacillus amyloliquefaciens* are efficient in controlling fire blight disease. *J. Biotechnol.* **2009**, *140*, 38−44.

(19) Koumoutsi, A.; Chen, X. H.; Vater, J.; Borriss, R. DegU and YczE positively regulate the synthesis of bacillomycin D by *Bacillus amyloliquefaciens* strain FZB42. *Appl. Environ. Microbiol.* **2007**, *73*, 6953−6964.

(20) Liu, J.; Zhou, T.; He, D.; Li, X.-z.; Wu, H.; Liu, W.; Gao, X. Functions of lipopeptides bacillomycin D and fengycin in antagonism of *Bacillus amyloliquefaciens* C06 towards *Monilinia fructicola*. *J. Mol. Microbiol. Biotechnol.* **2011**, *20*, 43−52.

(21) Ongena, M.; Jacques, P. Bacillus lipopeptides: Versatile weapons for plant disease biocontrol. *Trends Microbiol.* **2008**, *16*, 115−125.

(22) Lu, X. L.; Xu, Q. Z.; Liu, X. Y.; Cao, X.; Ni, K. Y.; Jiao, B. H. Marine drugs—Macrolactins. *Chem. Biodiversity* **2008**, *5*, 1669−1674.
(23) Yu, G. Y.; Sinclair, J. B.; Hartman, G. L.; Bertagnolli, B. L. Production of iturin A by *Bacillus amyloliquefaciens* suppressing *Rhizoctonia solani*. *Soil Biol. Biochem.* **2002**, *34*, 955−963.