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Screening and Identification of Antimicrobial Compounds from *Streptomyces bottropensis* Suppressing Rice Bacterial Blight

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***Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is the most devastating pathogen to *Oryza sativa* and has been shown to cause bacterial blight. Two bioactive compounds showing antimicrobial activities against *Xoo* strain KACC 10331 were isolated from a *Streptomyces bottropensis* strain. The ethyl acetate extract was fractionated on a Sephadex LH-20 column, and then purified by preparative HPLC. The purified compounds were identified as bottromycin A2 and dunaimycin D3S by HR/MS and ^{1}H NMR analyses. The MIC value against *Xoo* and the lowest concentration still capable of suppressing rice bacterial blight were 2 μg/ml and 16 μg/ml for bottromycin A2, and 64 μg/ml and 0.06 μg/ml for dunaimycin D3S, respectively. These two compounds were shown to exert different bioactivities *in vitro* and in rice leaf explants.**

Keywords: Bottromycin A2, dunaimycin D3S, *Streptomyces bottropensis*, *Xanthomonas oryzae* pv. *oryzae*, antibacterial activity, rice bacterial blight

Xanthomonas oryzae pv. *oryzae* (*Xoo*) causes rice bacterial blight (BB) and has been reported to result in a severe loss in yield (up to 50%) in tropical Asian countries [1]. Several genomic and genetic studies have been performed to better understand the interaction between pathogens and the host with the aim of developing rice that is broadly resistant against various *Xoo* strains. For instance, the whole genome map of *X. oryzae* pv. *oryzae* str. KACC 10331, which is a representatively virulent Korean strain, has been fully defined [12]. In order to control this plant disease, commercial chemicals have been employed in the fields; however, the world-wide use of artificial pesticides is gradually decreasing because of their excessive toxicity and degradation properties [2]. Thus, it is necessary to identify safe natural bioactive substances that can be used as alternatives to artificial pesticides. Biological control using microorganisms for inhibition of plant disease holds promise as a robust and long-lasting alternative to synthetic chemicals [7]. *Streptomyces* has been historically examined as a microorganism that can produce novel compounds that suppress pathogenic microbes. In recent studies, the antagonistic activities of *Streptomyces* against several *Xanthomonas* strains including *Xoo* were tested and confirmed [18, 23].

In this study, antimicrobial compounds produced by *Streptomyces bottropensis* strain MJM3389 against *X. oryzae* pv. *oryzae* KACC 10331 were identified. One was a cyclic peptide and the other was a spiroketal macrolide. Their potential bioactivities in regard to inhibiting rice bacterial blight were examined.

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Materials and Methods

Chemicals and Reagents

For the fermentation of *Xanthomonas oryzae* pv. *oryzae* KACC 10331 in M210 medium, sucrose and dipotassium phosphate (Junsei Chemical, Japan), NZ Amine (Sigma-Aldrich, MO, USA), and Bacto Yeast Extract (Becton Dickinson, MD, USA) were utilized. Mueller-Hinton broth was purchased from Becton Dickinson (MD, USA) and used to test the *in vitro* antibacterial activities of different samples. Sephadex LH-20 gel, which was used for column chromatography, was obtained from GE Healthcare (Sweden) and 2,4-diacetyphloroglucinol (DAPG) was purchased from Toronto Research Chemicals (Ontario, Canada). For LC/MS analysis, HPLC-grade water, Optima grade acetonitrile, and methanol were purchased from Fisher Scientific (PA, USA) and formic acid was obtained from Sigma-Aldrich (MO, USA). HPLC-grade water, acetonitrile, and methanol, which were used to purify active compounds, were purchased from Burdick & Jackson (MI, USA).

Bacterial Strains

Xanthomonas oryzae pv. *oryzae* KACC 10331 was provided from the Korean Agricultural Culture Collection (KACC, Suwon, Korea).

Streptomyces bottropensis MJM3389 was collected from soil in Imsil-gun (Jeounbuk, Korea), identified by sequence alignment of 16S rDNA, and deposited at ECUM (http://www.ecum.or.kr).

Preparation of *Xanthomonas oryzae* pv. *oryzae* KACC 10331

To activate *Xoo*, which was stored as a liquid stock in a deep-freezer, *Xoo* was placed on M210 agar and kept in a 28°C incubator for 24 h. A bacterial colony was picked and placed into 3 ml of M210 liquid medium in a 13 ml tube. The cells were then incubated at 28°C with shaking at 150 rpm for 24 h.

Fermentation and Identification of *Streptomyces bottropensis* MJM3389

One g of soil sample harvested from Imsil-gun (Jeounbuk, Korea) was washed out and then heated at 60°C for 30 min. The sample was placed on HV (Humic acid vitamin) agar medium to isolate *Streptomyces*, and the bacterial culture was coated on starch-casein KNO_3 agar (starch 1%, KNO_3 0.2%, K_2HPO_4 0.2%, NaCl 0.2%, casein 0.03%, $MgSO_4 \cdot 7H_2O$ 0.005%, $CaCO_3$ 0.002%, $FeSO_4 \cdot 7H_2O$ 0.001%, and agar 1.8%) and incubated at 28°C for 3 weeks. *Streptomyces* was fermented on 10 l of GSS medium (soluble starch 1%, glucose 2%, soybean meal 2.5%, beef extract 0.1%, yeast extract 0.4%, NaCl 0.2%, K_2HPO_4 0.025%, and $CaCO_3$ 0.2%, at pH 7.2) and divided into 650 ml in sixteen 2-l Erlenmeyer flasks. Fermentation was performed in shaking incubators by 200 rpm at 28°C for 7 days.

Full 16S rDNA of *Streptomyces* sp. MJM3389 was amplified using a universal primer set, 27F (5'-AGA GTT TGA TCA TGG CTC AG-3') and 1492R (5'-GGA TAC CTT GTT ACG ACT T-3') and the sequences were aligned on the NCBI homepage (National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov/).

Isolation and Purification of Active Compounds

Fermented *S. bottropensis* MJM3389 (10 l) was centrifuged and the supernatant was extracted with ethyl acetate. The extract was fractionated through Sephadex LH-20 column chromatography and eluted in 80% aqueous methanol [13]. The column diameter was 2.2 cm and the length was 110 cm. Two compounds from the active fraction were purified by preparative reverse-phase high-performance liquid chromatography (RP-HPLC) on a YMC-Pack Pro C_{18} column (250 × 4.6 mm). The HPLC system (Hitachi, Japan) consisted of diode array detector L-2455 and binary pumps of L-2130. The mobile phases consisted of water with 5% acetonitrile (A) and 100% acetonitrile (B) and a flow rate of 1 ml/min was used. During purification, B was gradually flowed and changed from 0 to 95% over 50 min. The mobile phase was then switched to 100% B, and maintained for 5 min. The 100% B was then reversed to 100% A for 5 min to wash and stabilize the column.

LC–ESI/MS Analysis

Samples were analyzed using an LC–MS/MS system (Varian, CA, USA) combined with a 212-LC Binary Solvent Delivery System, a MetaTherm HPLC Column Heater, a Prostar 410 AutoSampler, a Prostar 335 photodiode array detector, and a 500-ion trap mass spectrometer. A Chromsep C_{18} column (150 × 2.0 mm) was coupled and the flow rate was kept at 0.2 ml/min. The mobile phase was composed of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). Purification was initiated with 10% B for 2 min; the concentration of B was then increased linearly from 10% to 100% over 28 min, and was held at 100% B for 5 min. B was then linearly decreased to 10% and was maintained for 5 min. Mass spectra were obtained by electrospray ionization both in negative and positive ionization modes at the range of *m/z* 100–1,000. The MS^n analysis was conducted as described above.

Analysis Conditions for High-Resolution MS

Compound **1** (bottromycin A2) was analyzed by the Korea Basic Science Institute (KBSI, Chungbuk, Korea). A Shimadzu LC–ESI–IT–TOF/MS (Kyoto, Japan) was utilized in positive-ion mode at a mass resolution of 10,000 and scan range of 100–1,500 *m/z*. The analytical conditions were as follows: positive spray voltage, +3.0 kV; detector voltage, 1.65 kV; skimmer voltage, 9.0 V; pressure of TOF region, 1.5×10^{-4} Pa, and temperature, 40°C; ion source temperature, 200°C; trap cooling gas (Ar) flow rate, 94 ml/min; ion trap pressure, 1.8×10^{-2} Pa; collision gas (Ar) flow rate, 43 ml/min; ion accumulation time, 10 min; precursor ion selected width, 3.0 *m/z* units; and the selected time, 20 min. Data acquisition and analysis were performed using the LC Solution 3.0 software (Shimadzu, Kyoto, Japan). The Shimadzu Composition Formula Predictor was also used to verify the identifications.

To estimate the molecular mass of Compound **2** (dunaimycin D3S), the analysis was conducted by KBSI (Seoul, Korea) using a fast atom bombardment mass spectrometer (HR-FAB/MS) JMS-700 (Jeol, Japan) at an FAB energy of 6 keV, emission current of 5 mA, and acceleration voltage of 10 kV.

NMR Measurements

^{1}H NMR spectra were recorded on an Advance 600 (Bruker, USA) at 600 MHz by the National Center for Inter-University Research Facilities (NCIRF, Seoul, Korea). The NMR spectra were collected in chloroform-d_3.

***In Vitro* Assay**

Pre-cultured *X. oryzae* KACC 10331 was adjusted to an OD 600 value of 0.5, which was measured using a GENESYS 6 UV-Vis Spectrophotometer (Thermo Scientific, USA) and subsequently diluted 1:20 (v/v) in Mueller-Hinton broth medium. Two hundred µl of broth medium was placed into 96-well plates. To determine the minimal inhibitory concentrations (MICs) of the two active compounds, the serial dilution method was used at concentrations ranging from 0.5 to 128 µg/ml, as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) [5, 6]. The plates were incubated at 28°C for 24 h. The MIC value was the lowest concentration that shows an increase in bacterial growth under 2% as measured by the OD value on a EL808 Microplate Reader (BioTek Ins., USA). This value was calculated using the following formula:

Increase in bacterial growth (%) =

$$\left[1-\frac{(C_{24}{}^{a}-C_{0}{}^{b})-(T_{24}{}^{c}-T_{0}{}^{d})}{C_{24}{}^{a}-C_{0}{}^{b}}\right]\times 100$$

$^aC_{24}$, control (not treated), incubated for 24 h; $^bC_0$, control (not treated) at 0 h

$^cT_{24}$, treated, incubated for 24 h; $^cT_0$, treated at 0 h

Rice Leaf Explants Screening

Pre-cultured *X. oryzae* KACC 10331 was diluted in distilled water and the active compounds were serial-diluted 2-fold using the same method described for the *in vitro* assay. Whole rice leaves were cut into 15 cm length and their surface was sterilized with a diluted antiseptic solution. The leaves were rinsed in distilled water three times and then the rice leaf explants (about 4 × 4 mm^2) were placed into 96-well plates. The plates were incubated at 37°C for a week. Experiments were performed in triplicate.

Evaluation of Diseased Leaf Area %

After incubating the rice leaf explants with the active compounds for a week, the diseased leaf area % (DLA %) was evaluated by converting the explants' color into black and white mode using Adobe Photoshop CS2. The white area, which represents lesions infected with *Xoo*, was calculated using an Image analyzer obtained from the Web site http://chemist. hosting.paran.com/imgarea/. The least concentrations were determined to sustain diseased leaf areas under 5%.

RESULTS

Identification of *Streptomyces bottropensis* MJM3389

Streptomyces sp. MJM3389 was identified based on the phylogenetic taxonomy with sequence alignment of 16S rDNA and had a genetic closeness of over 99% to the *S. bottropensis* GU201851 strain. *Streptomyces* sp. MJM3389 was thus defined as *Streptomyces bottropensis* MJM3389.

Characterization of the Active Compounds

The ethyl acetate extract of *S. bottropensis* MJM3389 was fractionated through Sephadex LH-20 column chromatography, and then the active fraction was separated every single minute for an hour by preparative C_{18} RP-HPLC. The two active compounds (compounds **1** and **2**) were collected at 22 min and 32 min on HPLC. The molecular weight was analyzed by LC–ESI/MS and determined to be 822 for compound **1** and 907 for compound **2** based on the *m/z* values of $[M+H]^+$ 823, $[M-H]^-$ 821 and $[M+H]^+$ 908, $[M+FA-H]^-$ 952, respectively. The molecular formulas were determined to be $C_{42}H_{62}N_8O_7S$ (**1**) by $[M+H]^+$ 823.4540 from ESI–Q–TOF/MS and $C_{50}H_{85}NO_{13}$ (**2**) by $[M+H]^+$ 908.6101 from HR-FAB/MS. The theoretical HR/MS values of bottromycin A2 and dunaimycin D3S were $[M+H]^+$ 823.4540 and $[M+H]^+$ 908.6099. Using the ^1H NMR data at 600 MHz, the proton numbers and chemical shifts were compared to previously published studies. Based on these structures, compounds **1** and **2** were tentatively determined to be bottromycin A2 and dunaimycin D3S, respectively. Bottromycin A2 was identified based on a partial cyclic structure with the chain moiety by ^1H, ^{13}C NMR spectra and DEPT, and 2D NMR including COSY and COLOC experiments [10]. Dunaimycin D3S was reported to be a macrolide, which bore a sugar moiety at the C-8 position, and the structure was obtained by ^1H, ^{13}C NMR, DEPT, HMQC, HMBC, and COSY spectrums [9]. The structures and chemical properties of the two active compounds, which were determined to be bottromycin A2 and dunaimycin D3S, are shown in Tables 1 and 2.

In Vitro Antimicrobial Activity

An *in vitro* assay was conducted to screen the antimicrobial activities of Compound **1** (bottromycin A2) and Compound **2** (duniamycin D3S) against *Xanthomonas oryzae* pv. *oryzae* KACC 10331. Compound **1** was noticeably active and had a MIC value of 2 μg/ml. In contrast, Compound **2** was much less active and had a MIC value of 64 μg/ml (Table 3, Fig. 4.). DAPG was utilized as a positive control and MICs were determined as the concentration where growth was suppressed to under 2%.

Rice Leaf Explants Screening

The ability of Compound **1** (bottromycin A2) and Compound **2** (duniamycin D3S) to suppress bacterial blight in rice leaf explants containing *Xoo* culture media was examined in 96-well plates using the 2-fold serial dilution method. The efficacy was converted into diseased leaf area % (DLA %). DLA % has been used to measure the severity of plant disease [4, 8], and the infected area of the rice explants under treatment was estimated by first converting the image using Adobe Photoshop CS2 and then analyzing the image with an image analyzer program. Compound **2** (dunaimycin D3S) seemed to protect the rice explants from *Xoo* infection. However, Compound **1** predominantly suppressed the microbial survival rather than rice explants

Table 1. Mass spectral data of Compound **1** and Compound **2**.

Identification	LC-ESI/MS			HR/MS			Formula	UV λ_{max} (nm)	Ref.[c]
	t_R (min)[a]	$[M+H]^+$ (*m/z*)	MS^n fragmentation	$[M+H]^+$ (*m/z*)	Calc.[b] (*m/z*)	Error ppm			
Compound **1** (bottromycin A2)	12.8	823.5	476.7 > 391.5 > 141.1	823.4540[d]	823.4540	0.0	$C_{42}H_{62}N_8O_7S$	225	[20]
Compound **2** (dunaimycin D3S)	17.9	908.8	656.9 > 621.1 > 223.5	908.6101[e]	908.6099	0.2	$C_{50}H_{85}NO_{13}$	224, 255	[9]

[a]t_R, Retention time; [b]Calc., calculated mass; [c]Ref., reference; [d]analyzed by LC–ESI–Q–TOF/MS; and [e]analyzed by HR-FAB/MS.

Table 2. ^{1}H NMR assignments for Compound **1** and Compound **2** (600 MHz, $CDCl_3$).

Position	Compound **1**[a]		Position	Compound **2**[b]	
	δ_H	Multiplicity, Coupling constants		δ_H	Multiplicity, Coupling constants
1	3.95	1H, bs	2	6.11	1H, d
2	3.49	2H, m	3	6.80	1H, dd
	3.70	m	5	3.76	1H, d
5	3.54	2H, m	6	1.82	1H, m
	3.73	m	7	3.80	1H, m
6	1.66	2H, m	8	3.67	1H, m
	2.00	m	9	3.10	1H, s
7	2.49	1H, m	11	N.I.[c]	2H
8	1.12	3H, d	12	N.I.[c]	2H
9	4.09	1H, d	13	N.I.[c]	2H
11	7.68	1H, d	14	N.I.[c]	2H
12	2.40	1H, dd	15	2.13	2H, br m
13	2.80	1H, m	16	5.17	1H, m
14	0.72	3H, d	17	6.08	1H, dd
15	0.79	3H, d	20	2.78	2H, dd
17	6.98	1H, d		2.46	dd
18	4.61	1H, d	21	4.12	1H, dd
20	0.98	3H, bs	22	2.03	1H, m
21	0.98	3H, bs	23	5.24	1H, m
22	0.98	3H, bs	24	1.70	2H, m
25	3.91	1H, s	26	N.I.[c]	2H
27	0.98	3H, bs		N.I.[c]	
28	0.98	3H, bs	27	N.I.[c]	2H
29	0.98	3H, bs		N.I.[c]	
31	6.92	1H, d	28	1.57	1H, m
32	4.93	1H	29	4.13	1H, d
33	3.38	1H, s	30	1.62	2H, m
34	1.38	3H, d		1.22	m
36	7.35	1H, m	31	3.81	1H, s
37	7.31	1H, m	32	1.46	2H, m
38	7.20	1H, m	33	1.02	3H, t
39	7.31	1H, m	34	1.35	3H, d
40	7.35	1H, m	35	0.90	3H, d
42	8.26	1H, d	36	1.10	3H, s
43	5.58	1H, ddd	37	2.51	2H, m
46	7.67	1H, d	39	1.40	3H, s
47	7.20	1H, d	40	1.40	3H, s
49	2.92	2H, dd	41	0.82	3H, d
	3.11	dd	42	0.94	3H, d
51	3.72	3H, s	43	N.I.[c]	1H
			44	N.I.[c]	1H
			45	N.I.[c]	1H
			46	N.I.[c]	1H
			47	N.I.[c]	1H
			48	N.I.[c]	1H

Table 2. Continued.

Position	Compound **1**[a]		Position	Compound **2**[b]	
	δ_H	Multiplicity, Coupling constants		δ_H	Multiplicity, Coupling constants
			1'	4.83	1H, dd
			2'	1.95	2H, m
				1.67	m
			3'	1.90	2H, m
				1.65	m
			4'	2.68	1H, m
			5'	4.44	1H, m
			6'	1.30	3H, d
			7'	2.41	3H, bs
			8'	2.41	3H, bs

With reference to the identification of [a]bottromycin A2 [10] and [b]dunaimycin D3S [9].
[c]N.I., not identified protons showing indeterminate peaks from δ 1.43 ~ 2.37.

Table 3. Inhibitory activity of Compound **1** and Compound **2**.

Compound	MIC[a] (μg/ml)	
	In vitro	In rice explants
Compound **1** (bottromycin A2)	2	16
Compound **2** (dunaimycin D3S)	64	0.06
DAPG[b]	4	32

[a]Minimum inhibitory concentration,
[b]2, 4-Diacetyphloroglucinol as a positive control.

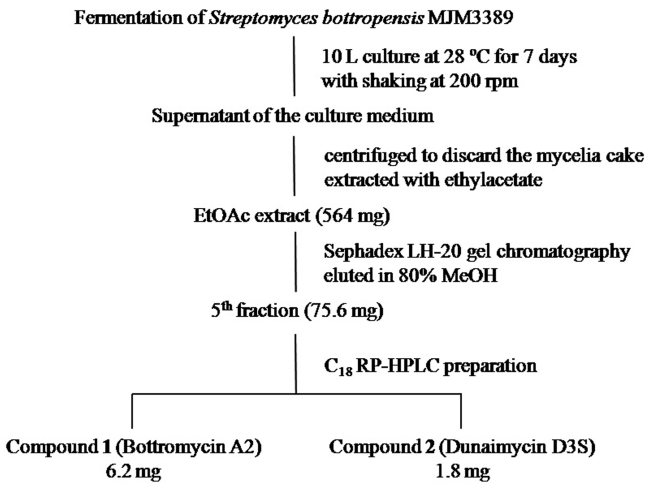

Fig. 1. Experimental scheme used to isolate Compound **1** (bottromycin A2) and Compound **2** (dunaimycin D3S) from *Streptomyces bottropensis* MJM3389.

protection from BB (Table 3, Fig. 5). DAPG was utilized as a positive control and the lowest concentrations were

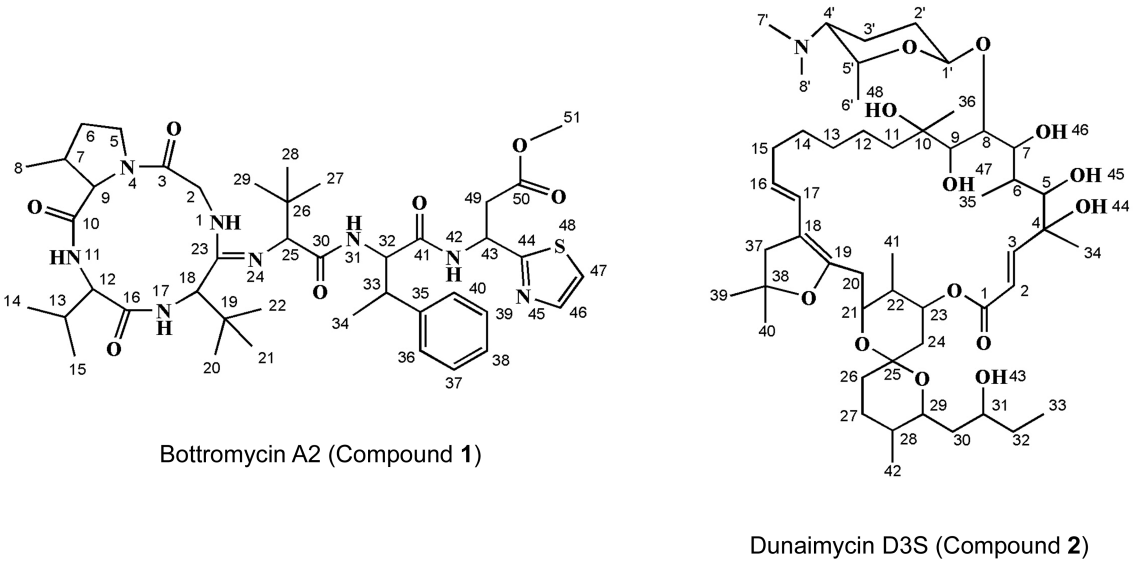

Fig. 2. Chemical structures of bottromycin A2 (Compound **1**) and dunaimycin D3S (Compound **2**).

determined where diseased leaf area was inhibited to under 10%.

DISCUSSION

The ethyl acetate extract of a *Streptomyces bottropensis* MJM3389, which was genetically closest to *S. bottropensis* GU201851, was shown to display bioactivity against *X. oryzae* pv. *oryzae* KACC 10331, and the isolates were identified as bottromycin A2 (Compound **1**) and dunaimycin D3S (Compound **2**). A cyclic peptide bottromycin A2 and a spiroketal macrolide dunaimycin D3S have been separately reported to be isolated from *S. bottropensis* and *S. diastatochromogenes*, respectively (Fig. 2) [11, 22]. It was not entirely unexpected that these two compounds were found in the same strain because the 16S rRNA sequences of *S. bottropensis* and *S. diastatochromogenes* showed 98.4% similarity [21]. Bottromycin A2 is known to be active against several bacteria and mycoplasma [16,

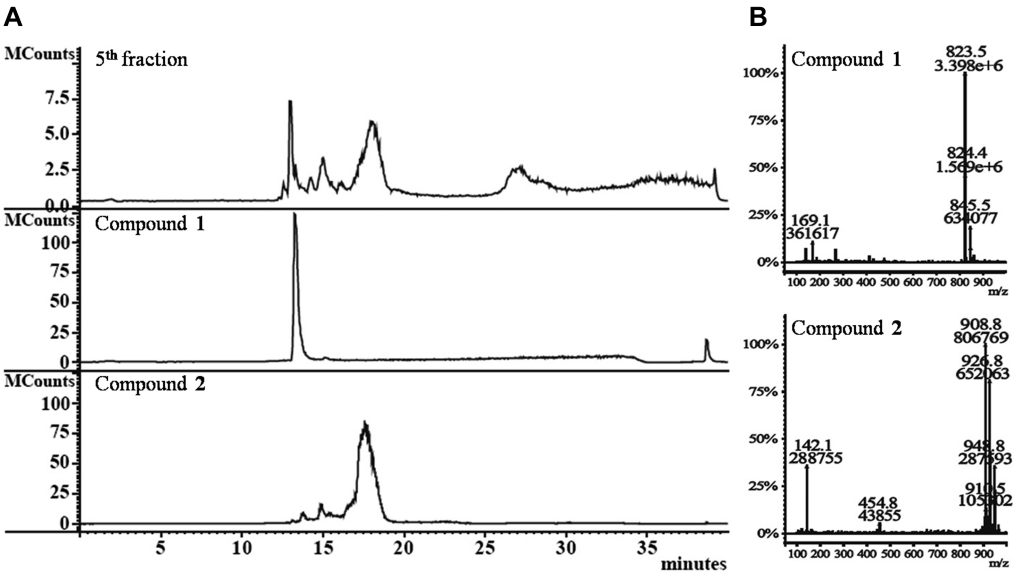

Fig. 3. Chromatograms and spectrums of Compound **1** (bottromycin A2) and Compound **2** (dunaimycin D3S) analyzed by LC–ESI/MS.
(**A**) Chromatograms of the active fraction eluted through Sephadex LH-20 gel chromatography, purified compounds **1** and **2** by C_{18} RP-HPLC preparation.
(**B**) Spectrums presented the *m/z* values of compounds **1** and **2** at positive mode.

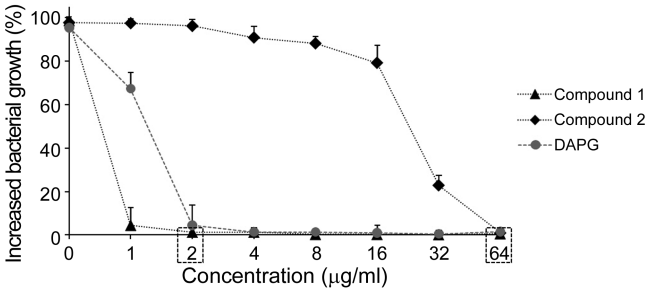

Fig. 4. *In vitro* antimicrobial activities of Compound **1** (bottromycin A2) and Compound **2** (dunaimycin D3S).
The activity of each compound was converted into how they inhibited the increase in bacterial growth as compared with 0 h OD and 24 h OD at 570 nm. Samples were treated with the different concentrations of the compounds using the 2-fold serial dilution method and the MICs were determined as the concentration where growth was suppressed to under 2%. The MIC values of compounds **1** and **2** were determined to be 2 μg/ml and 64 μg/ml, respectively. DAPG was employed as a positive control.

17, 22] and, dunaimycins, which are part of the 24-membered families, show antimicrobial, antifungal, and immunosuppressive activities [3, 11]. This is the first report that one strain of *Streptomyces* has antimicrobial activity against *Xanthomonas oryzae* pv. *oryzae* by producing two different antibiotics, bottromycin A2 and dunaimycin D3S. The bioactivities of these compounds were assayed using two different methods; an *in vitro* test to determine the minimum inhibitory concentration against *Xoo* and a rice leaf explants assay to measure the lowest concentration needed to suppress bacterial blight infection (least active concentration). Bottromycin A2 and dunaimycin D3S displayed a dual effect in the presence of rice leaf explants. Bottromycin A2 was effectively active with a minimum inhibitory concentration against *Xoo* of 2 μg/ml, which

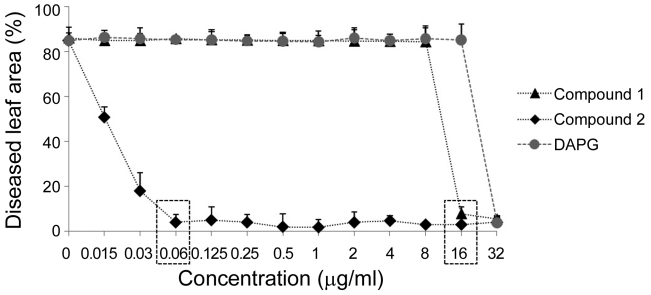

Fig. 5. Compound **1** (bottromycin A2) and Compound **2** (dunaimycin D3S) bioactivity against bacterial blight infection in rice leaf explants.
Rice leaf explants were treated with various compounds **1** and **2** concentrations using the 2-fold serial dilution method. The lowest concentrations needed to suppress the development of infected lesion by *X. oryzae* pv. *oryzae* KACC 10331 were determined to be 16 μg/ml for Compound **1** (bottromycin A2) and 0.06 μg/ml for Compound **2** (dunaimycin D3S), of which diseased leaf areas were under 10%. DAPG was employed as a positive control.

was much lower than the MIC value of dunaimycin D3S (64 μg/ml) (Fig. 4., Table 3). However, the *in vitro* activities were reversed in the rice leaf explants test. Interestingly, bottromycin A2 did not strongly act to suppress bacterial blight; instead, dunaimycin D3S exhibited a dramatically different activity with a least concentration of 0.06 μg/ml (Fig. 5, Table 3). Compared with the least active concentrations, bottromycin A2 had a stronger antimicrobial activity against *Xoo* but dunaimycin D3S was more effective in protecting the rice explants from bacterial blight infection. Thus, employing a mixture of bottromycin A2 and dunaimycin D3S will likely produce a much better effect against rice bacterial blight rather than using a single compound.

Bottromycin A2 has been reported to be an inhibitor of protein synthesis in bacterial systems [14]. It was shown to modulate peptidyl-tRNA on the *E. coli* ribosome not to be trans-located [15] and to prohibit A sites of polysomes in *E. coli* from proper functioning [19]. In addition, the 24-membered dunaimycins was reported to suppress Na^+ and K^+-ATPase in porcine cells. This effect was suggested to block crucial metabolites from crossing cell membranes; thus, dunaimycins could inhibit cell division [3]. However, the mechanism behind the *in vivo* activity of bottromycin A2 and dunaimycin D3S and why they displayed a dual effect is still unknown. Greenhouse tests with whole rice will be performed in further studies to examine whether those compounds actually function *in vivo*. In addition, the optimized fermentation conditions to maximize yield will need to be determined if bottromycin A2 and dunaimycin D3S are to be used as biological control agents against rice bacterial blight.

Acknowledgments

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References

1. Adhikari, T. B., T. W. Mew, and P. S. Teng. 1994. Progress of bacterial blight on rice cultivars carrying different Xa genes for resistance in the field. *Plant Dis*. **78:** 73–77.
2. Bajpai, V. K., N. T. Dung, H. J. Suh, and S. C. Kang. 2010. Antibacterial activity of essential oil and extracts of *Cleistocalyx operculatus* buds against the bacteria of *Xanthomonas* spp. *J. Am. Oil Chem. Soc.* **87:** 1341–1349.
3. Burres, N. S., U. Premachandran, A. Frigo, S. J. Swanson, K. W. Mollison, T. A. Fey, *et al*. 1991. Dunaimycins, a new complex of spiroketal 24-membered macrolides with immunosuppressive activity III. Immunosuppressive activities of dunaimycins. *J. Antibiot.* **44:** 1331–1341.

4. Campbell, C. L. and L. V. Madden. 1991. *Introduction to Plant Disease Epidemiology*, pp. 107–128.
5. Cui, C. B., M. Ubukata, H. Kakeya, R. Onose, G. Okada, I. Takahashi, *et al*. 1996. Acetophthalidin, a novel inhibitor of mammalian cell cycle, produced by a fungus isolated from a sea sediment. *J. Antibiot.* **49:** 216–219.
6. Dung, N. T., J. M. Kim, and S. C. Kang. 2008. Chemical composition, antimicrobial and antioxidant activities of the essential oil and the ethanol extract of *Cleistocalyx operculatus* (Roxb.) Merr and Perry buds. *Food Chem. Toxicol*. **46:** 3632–3639.
7. Emmert, E. A. B. and J. Handelsman. 1999. Biocontrol of plant disease: A (Gram-) positive perspective. *FEMS. Microbiol. Lett*. **171:** 1–9.
8. Forbes, G. A. and M. J. Jeger. 1987. Factors affecting the estimation of disease intensity in simulated plant structures. *Zeitschr. Pflanzenkrank. Pflanzensch.* **94:** 113–120.
9. Hochlowski, J. E., M. M. Mullally, G. M. Brill, and D. N. Whittern. 1991. Dunaimycins, a new complex of spiroketal 24-membered macrolides with immunosuppressive activity. II. Isolation and elucidation of structures. *J. Antibiotics*. **44:** 1318–1330.
10. Kaneda, M. 1992. Studies on bottromycins I. ^{1}H and ^{13}C NMR assignments of Bottromycin A2, the main component of the complex. *J. Antibiot*. **45:** 792–796.
11. Karwowski, J. P., M. Jackson, M. L. Maus, W. L. Kohl, P. E. Humphrey, and P. M. Tillis. 1991. Dunaimycins, a new complex of spiroketal 24-membered macrolides with immunosuppressive activity. I. Taxonomy of the producing organisms, fermentation and antimicrobial activity. *J. Antibiot.* **44:** 1312–1317.
12. Lee, B. M., Y. J. Park, D. S. Park, H. W. Kang, J. G. Kim, E. S. Song, *et al*. 2005. The genome sequence of *Xanthomonas oryzae* pathovar *oryzae* KACC 10331, the bacterial blight pathogen of rice. *Nucl. Acids Res*. **33:** 577–586.
13. Lim, C. S., J. Y. Kim, J. N. Choi, K. Ponnusamy, Y. T. Jeon, S. U. Kim, *et al*. 2010. Identification, fermentation, and bioactivity against *Xanthomonas oryzae* of antimicrobial metabolites isolated from *Phomopsis longicolla* S1B4. *J. Microbiol. Biotechnol.* **20:** 494–500.
14. Lin, Y. C. and N. Tanaka. 1968. Mechanism of action of bottromycin in polypeptide biosynthesis. *J. Biochem.* **63:** 1–7.
15. Lin, Y. C., T. Kinishta, and N. Tanaka. 1968. Mechanism of protein synthesis inhibition by bottromycin A2: Studies with puromycin. *J. Antibiot.* **21:** 471–476.
16. Nakamura, S., T. Chikaike, K. Karasawa, N. Tanaka, H. Yonehara, and H. Umezawa. 1965. Isolation and characterization of bottromycins A and B. *J. Antibiot*. **18:** 47–52.
17. Nakamura, S., T. Yajima, Y. Lin, and H. Umezawa. 1967. Isolation and characterization of bottromycin A2, B2, C2. *J. Antibiot*. **20:** 1–5.
18. Ndonde, M. J. M. and E. Sem. 2000. Preliminary characterization of some *Streptomyces* species from four Tanzanian soils and their antimicrobial potential against selected plant and animal pathogenic bacteria. *J. Microbiol. Biotechnol.* **16:** 595–599.
19. Otaka, T. and A. Kaji. 1983. Mode of action of bottromycin AZ: Effect of bottromycin polysomes. *FEBS Lett*. **153:** 53–59.
20. Takahashi, Y., H. Naganawa, T. Takita, H. Umezawa, and S. Nakamura. 1976. The revised structure of bottromycin A2. *J. Antibiot*. **29:** 1120–1123.
21. Takeuchi, T., H. Sawada, F. Tanaka, and I. Matsuda. 1996. Phylogenetic analysis of *Streptomyces* spp. causing potato scab based on 16S rRNA sequences. *Int. J. Syst. Bacteriol*. **46:** 476–479.
22. Waisvisz, J. M., M. G. V. D. Hoeven, J. V. Peppen, and W. C. M. Zwennis. 1957. Bottromycin. I. A new sulfur-containing antibiotic. *J. Am. Chem. Soc.* **79:** 4520–4521.
23. Yan Min, V., T. Da Quun, T. Shi Min, and Z. Ding. 2000. The antagonism of 26 strains of *Streptomyces* sp. against several vegetables pathogens. *Hebaei Agric. Univ*. **23:** 65–68.