JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Isolation and Antifungal Activity of 4-Phenyl-3-Butenoic Acid from *Streptomyces koyangensis* Strain VK-A60

JEE YEON LEE,† JUNG YEOP LEE,‡ SURK SIK MOON,§ AND BYUNG KOOK HWANG*,†

Division of Bioscience and Technology, College of Life and Environmental Sciences, Korea University, Seoul 136-713, Korea, Department of Medicinal Chemistry, College of Pharmacy and Life Sciences Institute, University of Michigan, 210 Washtenaw Ave, Ann Arbor, MI 48109, and Department of Chemistry, Kongju National University, Kongju 314-701, Korea

An antifungal compound was isolated from the culture broth of *Streptomyces koyangensis* strain VK-A60 using various chromatographic procedures. On the basis of the high-resolution EI-mass and ^{1}H and ^{13}C NMR data, the compound was identified as 4-phenyl-3-butenoic acid. *Colletotrichum orbiculare*, *Magnaporthe grisea*, and *Pythium ultimum* were most sensitive to 4-phenyl-3-butenoic acid. Strong inhibitory effects of 4-phenyl-3-butenoic acid also were found against *Pectobacterium carotovorum* subsp. *carotovorum* and *Ralstonia solanacearum*. 4-Phenyl-3-butenoic acid effectively suppressed the development of *M. grisea* on rice leaves at the concentration of more than 10 μg/mL, and the protective activity was in general similar to that of the commercial fungicide tricyclazole. Treatment with 100 μg/mL of 4-phenyl-3-butenoic acid also effectively inhibited the anthracnose development on cucumber plants, although its in vivo efficacy was somewhat less effective than that of the commercial fungicide chlorothalonil.

KEYWORDS: ***Streptomyces koyangensis*; 4-phenyl-3-butenoic acid; antifungal activity; cucumber anthracnose; rice blast**

INTRODUCTION

Microbial metabolites are expected to be potent fungicides to conquer the resistance and pollution likely caused by synthetic fungicides, because of their versatility in structure and activity (*1*, *2*) and biodegradable properties (*3*, *4*). A series of agrofungicides of microbial origin have been developed, such as blasticidin S (*5*), kasugamycin (*6*), polyoxin (*7*), validamycin (*8*), and mildiomycin (*9*). The microbial metabolites that were not evaluated by the previous screening procedures were often discovered for their potent antifungal activity against plant pathogenic fungi. Recently, the antibiotic compounds such as gopalamicin (*10*), tubercidin (*11*, *12*), manumycin-type antibiotics (*13*), rhamnolipid B (*14*), phenylacetic acid (*15*), phenazine-1-carboxylic acid (*16*), aerugine (*17*), and thiobutacin (*18*) were found to have potent antifungal activities for the control of some plant diseases.

The genus *Streptomyces* has been the richest source for all types of antibiotics. Substantial numbers of *Streptomyces* species or strains with a novel antibiotic activity still exist in nature (*19*). Accordingly, *Streptomyces* strains, as sources of a lager number and wider variety of new antibiotics, have been continuously noted rather than any other actinomycete genera. In our previous study, a number of actinomycete strains were isolated from soils of various regions in Korea (*20*). *Streptomyces koyangensis* sp. nov. strain VK-A60 (*21*) was isolated during the screening of actinomycete strains having antifungal activity against some plant pathogenic fungi.

The objectives of this study were to isolate and characterize antifungal substances active against plant pathogenic fungi produced by a novel *Streptomyces* species using various chromatographic procedures and NMR and mass spectral analysis and to evaluate its in vitro and in vivo antifungal activities against various plant pathogenic fungi. This is the first report to study 4-phenyl-3-butenoic acid (PBA) produced by *S. koyangensis* sp. nov. strain VK-A60 and its antifungal activity against plant pathogenic fungi.

MATERIALS AND METHODS

Production and Isolation of the Antifungal Compound. *Streptomyces koyangensis* strain VK-A60 (*21*) antagonistic to various plant pathogenic fungi was isolated from radish-growing soils collected from Ko-yang in Korea. The strain VK-A60 was precultured in 500 mL of yeast-malt extract broth (YMB) (10 g of malt extract, 4 g of yeast extract, 4 g of glucose, 20 g of agar, 1 L of H_2O, pH 7.3) in a 1-L Erlenmeyer flask on a rotary shaker at 150 rpm for 3 days at 28 °C. The 5-mL aliquot of the culture broth was transferred into 500 mL of glycerol dextrin broth (GDB) [20 g of glycerol, 20 g of dextrin, 10 g of soytone, 3 g of yeast extract, 2 g of $(NH_4)_2SO_4$, 4 g of K_2HPO_4, 1

* To whom correspondence should be addressed. Tel: +82-2-3290-3061. Fax: +82-2-925-1970. E-mail: bkhwang@korea.ac.kr.
† Korea University.
‡ University of Michigan.
§ Kongju National University.

10.1021/jf050957r CCC: $30.25
Published on Web 09/07/2005

L of H_2O, pH 7.0] in a 1-L Erlenmeyer flask. The inoculated flasks were incubated for 14 days at 28 °C on a rotary shaker at 150 rpm. The culture broth of strain VK-A60 (100 L) was centrifuged at 3200*g* for 20 min to remove mycelial mats. The resulting supernatants were purified by Diaion HP-20 column chromatography. The column was eluted with a stepwise gradient of water and methanol (0:100, 20:80, 40:60, 60:40, 80:20, 100:0, v/v). Each eluate (3 L) was concentrated to a small volume and bioassayed against *Colletotrichum orbiculare*, *Phytophthora capsici*, *Rhizoctonia solani*, and *Magnaporthe grisea* using a paper disk method. The 80% and 100% methanol eluates were highly active against *C. orbiculare* and *M. grisea*. The effective fractions were combined and partitioned with ethyl acetate. The solvent was subsequently evaporated using a rotary evaporator. The ethyl acetate phase was further purified by flash column chromatography of silica gels (silica gel $60F_{254}$, 63−200 μm, Merck). The crude extracts were loaded on an open glass column (150 × 200 mm) packed with silica gels. The column was eluted with stepwise gradients of chloroform and methanol (100:0, 90:10, 80:20, 70:30, 50:50, 30:70, 10:90, v/v). Each fraction was concentrated and bioassayed. The antifungal 100% and 90% chloroform fractions were combined and further purified by C_{18} reverse phase flash chromatography (Lichroprep RP-18, 40−63 μm, Merck, Darmstadt, Germany). The column was eluted with stepwise gradients of methanol and water (0:100, 20:80, 40:60, 60:40, 80:20 and 100:0, v/v). Each fraction of the eluate was concentrated in vacuo and antifungal activity against *C. orbiculare* and *M. grisea* was evaluated using the paper disk method. The antifungal 60% and 80% methanol fractions were combined and concentrated. The concentrates were dissolved in methanol and chromatographed on Sephadex LH-20 column (26 × 950 mm, C26/100 column packed with Sephadex LH-20 resin, Pharmacia, Upsala, Sweden). The Sephadex LH-20 column was eluted with methanol at a flow rate of 0.15 mL/min, and the fractions were collected using a fraction collector (Pharmacia Redifrac, Pharmacia). Among 250 fractions obtained from Sephadax LH-20 column chromatography, fraction no. 65-189 showed high activity against *C. orbiculare*. The pooled active fractions were subjected to HPLC with a C_{18} reverse phase column (SymmetryPrep C_{18} column, 7 μm, 7.8 × 300 mm, Waters). The chromatography was conducted with a Gilson HPLC system (Gilson, Middleton, WI) at a flow rate of 2 mL/min using a linear gradient solvent system from 20% acetonitrile to 80% acetonitrile in 10 mM ammonium hydroxide. The separation was monitored at absorbance of 250 nm by a UV−vis detector (118 UV−vis detector, 0.2 mm cell path, Gilson). Finally, the pure antifungal compound active against *C. orbiculare* was obtained from a single peak with the retention time of 3.23 min at 250 nm.

Structure Elucidation of the Antifungal Compound. The UV spectrum of the compound was recorded on Beckman DU 650 spectrometer (Beckman Instruments, Inc., Fullerton, CA). The electron impact mass spectrum was measured on JEOL JMS-700 Mstation mass spectrometer (JEOL, Tokyo, Japan). ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR, and distortionless enhancement by polarization transfer (DEPT) spectra were recorded on a Bruker AMX 500 NMR spectrometer (Bruker, Rheinstetten, Germany) using $CDCl_3$ with TMS as an internal standard. Two-dimensional NMR spectra, such as ^{1}H−^{1}H correlation spectroscopy (COSY), hetero single quantum coherence (HSQC), and heteronuclear correlation via multiple bond connectivities (HMBC), also were measured using the Bruker AMX 500 NMR spectrometer.

Detection of in Vitro Antimicrobial Activity. During purification procedures, the antifungal effects of each fraction were determined by paper disk bioassay method. The fractions obtained from each chromatography step were loaded onto sterile paper disks (8 mm in diameter). The molten potato dextrose agar (Difco) containing conidial and mycelial suspension of test fungi was poured into 9-cm Petri dishes. After solidification of seeded agar, completely desiccated paper disks loaded with the fractions were laid onto the center of the plate.

The minimum inhibitory concentrations (MICs) of purified antifungal compound against plant pathogenic oomycetes and fungi, yeast, and bacteria was determined in a 24-well microtiter dish (Cell Wall, Corning Glass Works, Corning) using a method modified from Nair et al. (*10*). The inocula used in this test were a zoospore suspension (10^6 zoospore/mL) of *P. capsici* and *Pythium ultimum*, a mycelial suspension of *R. solani*, and a spore suspension (10^6 spore/mL) of other plant pathogenic fungi: *Alternaria mali*; *Botrytis cinerea*; *Cladosporium cucumerinum*; *C. orbiculare*; *Cylindrocarpon destructans*; *Fusarium oxysporum* f.sp. *lycopersici*; *M. grisea*; *Sclerotinia sclerotiorum*. They were incubated on potato dextrose agar at 20−28 °C. Antimicrobial activities of yeasts (10^4 CFU/mL), *Candida albicans* and *Saccharomyces cerevisiae*, and bacteria (10^4 CFU/mL), *Bacillus subtilis* subsp. *subtilis*, *Pectobacterium carotovorum* subsp. *carotovorum*, *Ralstonia solanacearum*, and *Xanthomonas vesicatoria*, were also evaluated. A 10-μL suspension of spores or cells at each concentration was added to each well containing 1 mL of potato dextrose broth (Difco) or nutrient broth (Difco). The antifungal compound dissolved in methanol, ranging in the concentration from 0 to 100 μg/mL, was dispensed into microtiter plates. The plates inoculated with *B. cinerea*, *C. cucumerinum*, and the other microorganisms were incubated at 22−28 °C. The antimicrobial activity of the compound against the microorganisms was evaluated after incubation for 2−5 days by comparing with a positive control containing culture broth and microorganisms without the compound. The lowest concentrations of the compound where no growth of microorganisms was observed were considered to be MICs.

Evaluation of in Vivo Antifungal Activity. The protective activity of the antifungal compound from the cultures of the strain VK-A60 on cucumber plants against *C. orbiculare* was evaluated in a growth room. Cucumber (*Cucumis sativus* L., cv. Baekrokdadaki) seeds were sown in plastic pots (5 × 15 × 10 cm) containing a steam-sterilized soil mix [peat moss, perlite, and vermiculite (5:3:2, vol:vol:vol), sand and loam soil (1:1:1, vol:vol:vol)]. Cucumber plants were raised in a growth room at 28 ± 2 °C with 80 μmol photons m^{-2} s^{-1} (fluorescent lamps). The commercial fungicide chlorothalonil and the purified compound, dissolved in water and methanol, respectively, were diluted with 0.1% Tween 20 solution to give the concentrations of 10, 50, 100, and 500 μg/mL. Each of diluted chemical solutions was sprayed on the primary leaves of cucumber plants at the 3-leaf stage 1 day before inoculation with *C. orbiculare*. Conidial suspensions (10^6 conidia/mL) of *C. orbiculare* in 0.05% Tween 20 were uniformly sprayed on the leaves of cucumber plants. Control plants were sprayed with the solution of Tween 20 only. The inoculated cucumber plants were placed in a dew chamber at 28 ± 1 °C for 24 h and then transferred to the growth room. Lesions on the secondary leaves of cucumber plants were counted 6 days after inoculation. Data are the means of lesion number/cm^2 leaf area of 6 plants/treatment with 3 replicates.

The antifungal compound was evaluated in a greenhouse for the ability of suppressing leaf blast development on rice. Rice (*Oryza sativa* L., cv. Nakdong) was raised in plastic pots (5 × 15 × 10 cm) filled with the steam-sterilized soil. Plants were grown in a greenhouse, and fertilizer was applied to rice plants. The commercial fungicide tricyclazole was used to compare the antifungal activity with the compound. The antifungal compound and tricyclazole dissolved in methanol and water, respectively, were diluted with 0.1% Tween 20 to give the concentrations of 1, 10, 50, 100, and 500 μg/mL. Each of the chemical solutions was sprayed on the leaves of rice at the 8-leaf stage 1 day before inoculation with *M. grisea*. Conidial suspension (10^5 conidia/mL) of *M. grisea* was sprayed on the rice leaves until runoff. Control plants were sprayed with the solution of Tween 20 only. The inoculated plants were placed in a dew chamber for 24 h at 28 °C and then transferred to the greenhouse. Lesions on the leaves were counted 5 days after inoculation when typical lesions appeared on the leaves of plants. Data are the means of lesion number/cm^2 leaf area of 6 plants/treatment with 3 replicates.

Statistical Analysis. Statistical analyses were conducted with the Statistical Analysis System for personal computers (SAS Institute, Cary, NC). Fisher's protected least significant difference (LSD) at $P = 0.05$ was applied to determine whether differences between treatments were significant.

4-Phenyl-3-Butenoic acid ($C_{10}H_{10}O_2$): HREIMS *m*/*z* 162.0680 [M^+] (theor *m*/*z* 162.06806); ^{1}H NMR (CD_3OD, 500 MHz) δ 7.39 (1H, d, J = 8.5, H-2 and H-6), 7.29 (1H, t, J = 8.5, H-3 and H-5), 7.20 (1H, t, J = 8.5, H-4), 6.50 (1H, d, J = 16.0, H-7), 6.35 (1H, dt, J = 16.0; 7.0, H-8) and 3.22 (2H, d, J = 7.0, H-9); ^{13}C NMR (CD_3OD, 125 MHz) δ 176.0 (C-10), 138.6 (C-1), 134.0 (C-7), 129.5 (C-3 and C-5), 128.4 (C-4), 127.2 (C-2 and C-6), 123.9 (C-8) and 39.5 (C-9).

RESULTS AND DISCUSSION

Production and Isolation of the Antifungal Compound. Optimal medium and culture time for producing antibiotics from *Streptomyces koyangensis* VK-A60 were examined to determine the conditions for a large-scale production of antibiotics. The strain VK-A60 was cultured at 28 °C for 4−14 days in the four media containing different carbon and nitrogen sources, such as GDB, glycerol peptone broth (GPB) (20 g of glycerol, 10 g of polypeptone, 5 g of meat extract, 1 L of H_2O, pH 7.0), starch casein broth (SCB) (20 g of soluble starch, 0.6 g of tryptone peptone, 4 g of K_2HPO_4, 4 g of KNO_3, 4 g of NaCl, 1 g of $MgSO_4 \cdot 7H_2O$, 20 mg of $FeSO_4 \cdot 7H_2O$, 40 mg of $CaCO_3$, 1 L of H_2O, pH 7.0), and starch glucose broth (SGB) (20 g of soluble starch, 10 g of glucose, 5 g of yeast extract, 5 g of casamino acid, 1 L of H_2O, pH 7.0). The strain VK-A60 grew well in most media such as SGB, GDB, and GPB, except for SCB. The growth of strain VK-A60 reached an idiophase 12 days after inoculation in GDB. Production of antibiotics by the strain VK-A60 in GDB distinctly occurred after incubation for 4 days, with a maximum level at 14 days after incubation, which indicated that the strain VK-A60 require a relatively long period to produce antibiotics in GDB, compared with other *Streptomyces* spp. In this study, addition of the glycerol as a carbon source in GDB remarkably accelerated the production of antibiotic compounds from the cultures of the strain VK-A60. It is interesting to note that glycerol is an important nutritional factor for a large-scale production of antibiotic compounds in the culture media. It seems likely that involvement of glycerol in GDB may limit the vegetative growth of the strain VK-A60 but significantly accelerate the biosynthesis of antibiotic compounds.

As long as microorganisms grow in triphosphase, metabolites are available as basic cell constituents. However, at the beginning of the idiophase when cell multiplication ceases, the microorganism begins to produce secondary metabolites, which show various biological functions, including antibiotic activity (*22*). The kinds or quantities of targeted antibiotics may vary according to the components of media or cultural conditions. Although *S. koyangensis* strain VK-A60 grew slowly on the GDB broth relative to the other several media tested, the GDB broth was found to be most favorable for production of antifungal antibiotics.

The antifungal compound was isolated from the fermentation broth of *S. koyangensis* strain VK-A60 using various chromatographic procedures. A total of 7.8 mg of pure compound, active against *C. orbiculare*, was obtained from a single peak by preparative HPLC. The antifungal compound was soluble in organic solvents such as methanol, ethanol, and ethyl acetate but poorly soluble in water.

Structure Elucidation of the Antifungal Compound. The structure of the antifungal compound was determined by analyzing NMR and mass spectral data. The EI mass spectrum of the compound confirmed its molecular weight to be 162. The major peak at *m*/*z* 117 in the mass spectrum reflects the molecular ion split out of a carboxyl fragment. The molecular formula of compound was deduced as $C_{10}H_{10}O_2$ on the basis of the analysis of high-resolution EI mass spectroscopy, which showed a molecular ion peak at *m*/*z* 162.0680 (M^+) (data not shown). From the analysis of ^{1}H NMR spectrum, the presence of two double-bonded methine groups was confirmed at the positions of δ 7.20, 7.29, and 7.39. Aromatic carbons were also observed at the position of δ 127.2, 128.4, 129.5, and 138.6. A methine group was confirmed at the position of δ 6.35 and 6.50 of ^{1}H NMR spectrum, which was ascertained at the carbon resonance at δ 123.9 and 134.0. The methylene group was detected at δ 3.22 in the ^{1}H NMR spectrum and δ 39.5 in the ^{13}C NMR spectrum, respectively. The ^{13}C NMR spectrum indicated the presence of a carboxyl group at δ 176.0. The correlations of methine proton (δ 6.50, H-7) with other methine proton (δ 6.35, H-8) were confirmed by the ^{1}H−^{1}H COSY spectral analysis. The COSY spectrum also showed the correlations of the methine proton (δ 6.35, H-8) with the methylene proton (δ 3.22, H-9). The possible connectivity between aromatic proton (δ 7.39, H-2) and methine proton (δ 6.35, H-8) was confirmed by the analysis of NOESY spectrum. The NOESY spectrum also showed the correlations of the aromatic proton (δ 7.39, H-6) with methine proton (δ 6.50, H-7). On the basis of all the spectral data, the structure of the antifungal compound was determined to be *trans*-4-phenyl-3-butenoic acid (molecular formula $C_{10}H_{10}O_2$).

4-Phenyl-3-butenoic acid (PBA) was first reported to be effective against *Trichomonas vaginalis* (*23*). PBA shows significant activity against aspen decay fungus *Phellinus tremulae* at a concentration as low as 1 μg/mL. Trifonov et al. (*24*) reported the inhibitory activity of homologues and analogoues of PBA against *P. tremulae*. Peptidylycine-α-hydroxylating monooxygenase (PHM) catalyzes the first and rate-limiting reactions in the two-step process in α-amidates neural and endocrine peptides. Mueller et al. (*25*) found that PBA acts a potent mechanism-based inhibitor of PHM in vivo. The effect of PBA on PHM activity in living systems is not well characterized. However, Ogonowski et al. (*26*) and Muller et al. (*25*) showed that the inhibitory effect of PBA in cardiac atrium, pituitary, and brain of rats. PBA and the structural analogue are known as a potent inhibitor of peptidylglycine amidating monooxygenase (PAM) reducing the rate of enzyme conversion (*27*) and a modulator of the activity of γ-glutamic acid-transpepidase as well as an antibacterial agent (*28*). Because of some harmful effects of PBA on mammals as described above, further studies on biological activities of the analogous compounds of PBA should be done to use it as a lead for the development of a fungicidal potential against plant pathogens.

It was recently shown that PBA was produced by *Streptomyces flavescens* B-2223 by Kolomiets et al. (*29*). They demonstrated that *S. flavescens* B-2223 had antibacterial potential against plant pathogenic bacteria belonging to *Pseudomonas*, *Erwinia*, and *Xanthomonas* and this antibacterial activity was associated with the production of PBA. However, there is little information about the isolation and antifungal activity of PBA from other microorganisms. To our knowledge, this is the first report to study that *S. koyangensis* sp. nov. strain VK-A60 produces PBA active against various microorganisms including plant pathogenic fungi.

In Vitro and in Vivo Antifungal and Antimicrobial Activities of 4-Phenyl-3-Butenoic Acid. In vitro antimicrobial spectrum of the PBA was determined in microtiter plates using a serial dilution method. PBA showed inhibitory effect against mycelial growth of *A. mali*, *C. cucumerinum*, *C. orbiculare*, *C. destructans*, *M. grisea*, *P. capsici*, *P. ultimum*, and *R. solani* but not against *B. cinerea*, *F. oxysporum* f.sp. *lycopersici*, and *S. sclerotiorum* (**Table 1**). In particular, PBA completely inhibited growth of *C. orbiculare*, *M. grisea*, and *P. ultimum* in the range 0.5−10 μg/mL. The growth of *C. albicans* and *S. cerevisiae* was not inhibited even at the concentration of 100 μg/mL. Inhibitory effects of PBA against bacteria, such as *B. subtilis* subsp. *subtilis*, *P. carotovorum* subsp. *carotovorum*, *R. solanacearum*, and *X. vesicatoria* was found at very low concentrations from 0.5 to 10 μg/mL.

Table 1. Minimum Inhibitory Concentrations (MICs) of 4-Phenyl-3-Butenoic Acid (PBA) from *Streptomyces koyangensis* Strain VK-A60 against Various Microorganisms Including Plant Pathogenic Fungi and Oomycetes, As Determined by the Microtiter Broth Dilution Method

microorganism	MIC (μg/mL)[a]
Alternaria mali	30
Botrytis cinerea	>100[b]
Cladosporium cucumerinum	30
Colletotrichum orbiculare	3
Cylindrocarpon destructans	30
Fusarium oxysporum f.sp. *lycopersici*	>100
Magnaporthe grisea	10
Phytophthora capsici	30
Pythium ultimum	0.5
Rhizoctonia solani	30
Sclerotinia sclerotiorum	>100
Candida albicans	>100
Saccharomyces cerevisiae	>100
Bacillus subtilis subsp. *subtilis*	3
Pectobacterium carotovorum subsp. *carotovorum*	1
Ralstonia solanacearum	0.5
Xanthomonas vesicatoria	10

[a] The lowest concentration of PBA required for complete inhibition of microbial growth. [b] The value of >100 represents that growth of the microorganisms was not completely inhibited at 100 μg/mL of PBA.

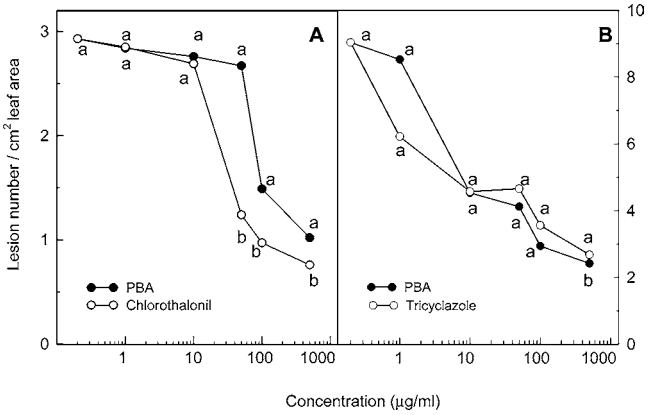

Figure 1. Effects of 4-phenyl-3-butenoic acid and the commercial fungicides chlorothalonil and tricyclazole on the development of (A) anthracnose caused by *Colletotrichum orbiculare* and (B) blast diseases caused by *Magnaporthe grisea* on the cucumber and rice leaves, respectively. Solutions of each compound were sprayed on the cucumber and rice plants 1 day before inoculation. Numbers of lesions on the second leaves of cucumber plants were rated on day 6 after inoculation. Numbers of lesion on the leaves of rice plants at the 7-leaf stage also were rated on day 5 after inoculation. Means at each concentration followed by the same letters are not significantly different ($P = 0.05$) according to the least significant difference test.

PBA and the commercial fungicide chlorothalonil were compared in the control of anthracnose development on leaves of cucumber plants (**Figure 1A**). At the 2-leaf stage, treatment of cucumber leaves with 10 μg/mL of PBA and chlorothalonil did not inhibit anthracnose development. However, the development of *C. orbiculare* on cucumber leaves was greatly suppressed by PBA at 100 μg/mL. A few typical lesions of *C. orbiculare* appeared on the cucumber leaves treated with the two chemicals at 500 μg/mL. The in vivo efficacy of PBA for the control of leaf blast in rice plants at the 8-leaf stage was evaluated under the greenhouse conditions (**Figure 1B**). The typical symptoms of the rice blast began to appear on rice leaves 4 days after inoculation. As the concentration of PBA and commercial fungicide tricyclazole increased, *M. grisea* infection was inhibited on the rice leaves. Treatment with 10 μg/mL of PBA and tricyclazole reduced lesion development on rice plants. Protective activity of PBA against *M. grisea* infection was in general similar to that of trycyclazole. PBA showed significantly protective activity against infection by *M. grisea* on rice leaves treated with 500 μg/mL.

The in vivo assay of PBA on plants is the most accurate means of predicting the potential antifungal activity in the fields, which reflect the intrinsic potency of the antibiotics, its chemical and physical properties, and stability in agricultural environment and distribution in host plants. Taken together, we conclude that 4-phenyl-3-butenoic acid from a novel *S. koyangensis* strain VK-A60 has not only potent in vitro antifungal and antioomycete activities against some plant pathogens but also is very effective for control of plant diseases, such as the cucumber anthracnose and rice blast diseases. For practical applications, further detailed study is required for evaluation of disease control efficacy in the field and the design of application strategies.

ACKNOWLEDGMENT

We thank the Korea Basic Science Institute for mass and NMR spectroscopy.

LITERATURE CITED

(1) Porter, N. Physico chemical and biophysical panel symposium biologically active secondary metabolites. *Pestic. Sci.* **1985**, *16*, 422−427.

(2) Lange, L.; Lopez, C. S. Microorganisms as a Source of Biologically Active Secondary Metabolites. In *Crop Protection Agents from Nature: Natural Products and Analogues*; Copping, L. G., Ed.; The Royal Society of Chemistry: London, 1996; pp 1−26.

(3) Tanaka, Y. T.; Omura, S. Agroactive compounds of microbial origin. *Annu. Rev. Microbiol.* **1993**, *47*, 57−87.

(4) Yamaguchi, I. Pesticides of Microbial Origin and Applications of Molecular Biology. In *Crop Protection Agents from Nature: Natural Products and Analogues*; Copping, L. G., Ed.; The Royal Society of Chemistry: London, 1996; pp 27−49.

(5) Takeuchi, S.; Hirayama, K.; Ueda, K.; Sakai, H.; Yonehara, H. Blasticidin S, a new antibiotic. *J. Antibiot.* **1957**, *11*, 1−5.

(6) Umezawa, H.; Okami, Y.; Hashimoto, T.; Suhara, Y.; Hamada, M.; Takeuchi, T. A new antibiotic kasugamycin. *J. Antibiot.* **1965**, *18*, 101−103.

(7) Isono, K.; Nagatsu, J.; Kobinata, K.; Sasaki, K.; Suzuki, S. Studies on Polyoxins, antifungal antibiotics. Part I. Isolation and characterization of polyoxin A and B. *Agric. Biol. Chem.* **1965**, *29*, 848−854.

(8) Iwasa, T.; Higashide, E.; Yamamoto, H.; Shibata, M. Studies on validamycins, new antibiotics II. Production and biological properties of validamycin A and B. *J. Antibiot.* **1970**, *23*, 595−602.

(9) Harada, S.; Kishi, T. Isolation and characterization of mildiomycin, a new nucleocide antibiotic. *J. Antibiot.* **1978**, *31*, 519−524

(10) Nair, M. G.; Chandra, A.; Thorogood, D. L. Gopalamicin, an antifungal macrolide produced by soil actinomycete. *J. Agric. Food Chem.* **1994**, *42*, 2308−2310.

(11) Hwang, B. K.; Ahn, S. J.; Moon, S. S. Production and Purification and antifungal activity of the antibiotic nucleoside, tubercidin, produced by Streptomyces. *Can. J. Bot.* **1994**, *72*, 480−485.

(12) Hwang, B. K.; Kim, B. S. In vivo efficacy and in vitro activity of tubercidin, an antibiotic nucleoside, for control of Phytophthora capsici blight in Capsicum annum. *Pestic. Sci.* **1995**, *44*, 255−260.

(13) Hwang, B. K.; Lee, J. Y.; Kim, B. S.; Moon, S. S. Isolation, structure elucidation, and antifungal activity of manumycin-type antibiotic from *Streptomyces flaveus*. *J. Agric. Food Chem.* **1996**, *44*, 3653−3657.

(14) Kim, B. S.; Lee, J. Y.; Hwang, B. K. In vivo control and in vitro antifungal activity of rhamnolipid B, a glycolipid antibiotic, against Phytophthora capsici and Colletotrichum orbiculare. *Pest Manage. Sci.* **2000**, *56*, 1029−1035.

(15) Hwang, B. K.; Lim, S. W.; Kim, B. S.; Lee. J. Y.; Moon, S. S. Isolation and In vivo antifungal activity of phenylacetic acid and sodium phenylacetate from Streptomyces humidus. *Appl. Environ. Microbiol.* **2001**, *67*, 3739−3645.

(16) Lee, J. Y.; Moon, S. S.; Hwang, B. K. Isolation and in vitro and in vivo activity against Phytophthora capsici and Colletotrichum orbiculare of phenazine-1-carboxylic acid from *Pseudomonas aeruginosa* strain GC−B26. *Pest Manage. Sci.* **2003**, *59*, 872−882.

(17) Lee, J. Y.; Moon, S. S.; Hwang, B. K. Isolation and antifungal and antioomycete activities of aerugine produced by *Pseudomonas fluorescens* strain MM-B16. *Appl. Environ. Microbiol.* **2003**, *69*, 2023−2031.

(18) Lee, J. Y.; Moon, S. S.; Yun, B. K.; Yoo, I. D.; Hwang, B. K. Thiobutacin, a novel antifungal and antioomycete antibiotic from Lechevalieria aerocolonigenes. *J. Nat. Prod.* **2004**, *67*, 2076−2078.

(19) Okami, Y.; Hotta, K. Search and Discovery of New Antibiotics. In *Actinomycetes in Biotechnology*; Goodfellow, M., Williams, S. T., Mordarski, M., Ed.; Academic Press: London, 1988; pp 33−67.

(20) Lee, J. Y.; Hwang, B. K. Diversity and isolation of antifungal actinomcetes in various vegetative soils of Korea. *Can. J. Microbiol.* **2002**, *48*, 407−417.

(21) Lee, J. Y.; Lee, J. Y.; Jung, H. W.; Hwang, B. K. Streptomyces koyangensis sp.nov., a novel actinomycete producing 4-phenyl-3-butenoic acid. *Int. J. Syst. Evol. Microbiol.* **2005**, *55*, 257−262.

(22) Woodruff, H. B. Natural products from microorganisms. *Science* **1988**, *208*, 1225−1229.

(23) Khristov, K. Effect of antiamino acids on Trichomonas vaginalis. *Rev. Biol. Trop.* **1973**, *21*, 187−195.

(24) Trifonov, L. S.; Ayer, W. A.; Chakaravarty, P.; Hiratsuka, Y. Structure−activity relationships of congeners of 4-phenyl-3-butenoic acid against aspen decay and strain fungi. *Eur. J. For. Pathol.* **1996**, *26*, 273−280.

(25) Mueller, G. P.; Driscoll, W. J.; Eipper, B. A. In vivo inhibition of peptidylating monooxygenase by 4-phenyl-3-butenoic acid. *J. Pharmacol. Exp. Ther.* **1999**, *290*, 1331−1336.

(26) Ogonowski, A. A.; May, S. W.; Moore, A. B.; Barrett, L. T.; O'Bryant, C. L.; Pollock, S. H. Antiinflammatory and analgesic activity of an inhibitor of neuropeptide amidation. *J. Pharmacol. Exp. Ther.* **1997**, *280*, 846−853.

(27) Bradbury, A. F.; Mistry, J.; Roos, B. A.; Smyth, D. G. 4-Phenyl-3-butenoic acid, and in vivo inhibitor of peptidylglycine hydrolase (peptide amidating enzyme). *Eur. J. Biochem.* **1990**, *189*, 363−370.

(28) Thomson, G. A.; Meister, A. Modulator of γ-glutamyl transpepidase activities by hippurate and related compounds. *J. Biol. Chem.* **1980**, *255*, 2109−2113.

(29) Kolomiets, E. I.; Zdor, N. A.; Romanovskaya, T. V. Investigation of antibacterial metabolites produced by Streptomyces flavescens B-2223. Bull. *Pol. Acad. Sci.: Biol. Sci.* **2003**, *51*, 67−72.

Received for review April 25, 2005. Revised manuscript received July 11, 2005. Accepted July 13, 2005. This research was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund) (KRE-2003-214-C00118) to Jung Yeop Lee.

JF050957R