Isolation, Structure Elucidation, and Antifungal Activity of a Manumycin-Type Antibiotic from *Streptomyces flaveus*

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A manumycin-type antibiotic, SW-B, has been isolated from the solid agar culture of *Streptomyces flaveus* strain A-11. The structure was determined by mass spectrometry and one- and two-dimensional NMR spectroscopy. SW-B was detected at R_f 0.5 on the TLC plate developed with chloroform−methanol (90:10 v/v). The UV spectrum of SW-B in methanol showed peaks at 208 and 260 nm. The antibiotic SW-B was confirmed to be a derivative of a manumycin-type antibiotic, 2,4,6-trimethyldeca-(2*E*,4*E*)-dienamide ($C_{13}H_{23}NO$, 209.1780). SW-B showed strong antifungal activity against *Phytophthora capsici, Magnaporthe grisea, Cladosporium cucumerinum*, and *Alternaria mali*. However, it lacked antimicrobial activity against yeast and bacteria.

INTRODUCTION

Since streptomycin was found to be active against some plant pathogenic bacteria, many antibiotics used in medicine have been finding use in agriculture. The intensive use of synthetic fungicides for control of plant diseases has created problems such as fungicide resistance and many environmentally detrimental side effects (Cohen and Coffey, 1986). However, antibiotics of microbial origin have few side effects on the environment and show little toxicity to host plants (Tanaka and Omura, 1993). They also possess selective activity against plant pathogens and are decomposed quickly. Therefore, toxicity in soils is not an issue (Mirsa, 1986). Agricultural antibiotics such as blasticidin S (Takeuchi et al., 1957), kasugamycin (Umezawa et al., 1965), polyoxin (Isono et al., 1965), and validamycin (Iwasa et al., 1970) are being used practically for the control of economically important plant diseases.

In general, microorganisms, especially actinomycetes, produce antibiotics that have various biological activities (Okami and Hotta, 1988). In particular, antifungal compounds have been reported increasingly year by year (Miyadoh, 1993). A variety of antifungal compounds have been produced from various actinomycetes. Deisovalerylblastmycin (Ishiyama et al., 1976) and gopalamicin (Nair et al., 1994) from *Streptomyces*, anthracycline antibiotics spartanamicins A and B (Nair et al., 1992) from *Micromonospora*, mildiomycin (Harada and Kichi, 1978) from *Streptoverticillium*, setamycin (Omura et al., 1981), and cystargin (Uramoto et al., 1988) from *Kitasatosporia* have well documented for antifungal activity.

It has long been known that some actinomycete strains of the same species could produce different antibiotics, whereas some strains belonging to different species produced the same antibiotics (Lechevalier, 1975). Antibiotic production by actinomycetes, therefore, may not be species-specific but strain-specific. Antibiotics of actinomycete origin show wide varieties of chemical structures encompassing aminoglycosides, anthracyclines, glycopeptides, β-lactams, macrolides, nucleosides, peptides, polyenes, polyethers, and tetracyclines (Okami and Hotta, 1988).

The manumycin group is a small and discrete class of antibiotics, which includes about a dozen secondary metabolites, all of them produced by *Strepromyces* species (Sattler et al., 1993). Manumycin, first reported by Buzzetti et al. (1963), bears two unsaturated carbon chains linked in "southern" and "eastern" orientation to a multifunctional six-membered ring (mC_7N unit) and a 2-amino-3-hydroxycyclopent-2-enone (C_5N unit) positioned at the end of the "southern" polyene chains (Sattler et al., 1993). Most of the manumycin group antibiotics exhibit a biological activity against Gram-positive bacteria, as well as antifungal and cytotoxic activities. Insecticidal effects and inhibition of polymorphonuclear leucocyte elastase were also found with manumycin A (Zeeck et al., 1987).

In the present study, the antibiotic SW-B was isolated from cell-free culture broth of *Streptomyces flaveus* by various purification procedures. The structure and antifungal activity of the antibiotic SW-B were determined by spectral techniques and bioassay, respectively. The antibiotic SW-B was found to be a derivative of the manumycin-type antibiotic 2,4,6-trimethyldeca-(2*E*,4*E*)-dienamide ($C_{13}H_{23}NO$, 209.1780). This compound showed strong inhibitory activity against some plant pathogenic fungi.

EXPERIMENTAL PROCEDURES

Production of the Antibiotic SW-B. *S. flaveus* strain A-11 antagonistic to various plant pathogenic fungi (Ahn and Hwang, 1992) was lawned onto Petri dishes containing yeast extract−peptone agar (25 g of glycerol, 10 g of yeast extract, 5 g of peptone, 3 g of CaCO₃, 2 g of NaCl, 0.5 g of MgSO₄·7H₂O, 0.5 g of K₂HPO₄, 20 g of agar, and 1 L of H₂O, pH 7.0) for 20 days at 28 °C. Hyphal mats were harvested from 1500 plates and extracted with methanol (30 L). Methanol extracts were concentrated *in vacuo* using a rotary evaporator (Büchi) and freeze-dried.

Isolation of the Antibiotic SW-B. Freeze-dried culture filtrates (120 g) of *S. flaveus* strain A-11 were dissolved in 200

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mL of methanol and chromatographed on a silica gel (60 F$_{254}$, 70–230 mesh, Merck) column (7 × 70 cm). This silica gel column was eluted at a flow rate of 1 mL min^{-1} using stepwise gradients of butanol–methanol (90:10, 1.5 L), butanol–methanol (60:40, 1.5 L), butanol–methanol (40:60, 1.5 L), butanol–methanol (10:90, 1.5 L), and methanol (1.5 L). Each eluate obtained from silica gel column chromatography was concentrated *in vacuo*. Antifungal activity of the eluates against *Phytophthora capsici* and *Magnaporthe grisea* was examined using the paper disk method. Eluates of butanol–methanol (90:10, 1.5 L) and butanol–methanol (60:40, 1.5 L), which showed strong antifungal activity against *P. capsici* and *M. grisea,* were chromatographed further on a Sephadex LH-20 column (5 × 90 cm). The Sephadex LH-20 column was eluted with methanol at 0.4 mL min^{-1} flow rate. The 5 mL fractions were collected using a fraction collector (Pharmacia RediFrac, Uppsala, Sweden). The antifugal activity of all 500 fractions was determined for *P. capsici* and *M. grisea.* Among silica gel fractions of butanol–methanol (90:10, 1.5 L), fractions 61–76 (SW-A), 81–116 (SW-B), 121–156 (SW-C), and 161–176 (SW-D) were active against both fungi. Among silica gel fractions of butanol–methanol (60:40, 1.5 L), fraction 116–146 (SW-E) was active against the two fungi. These were collected and then concentrated. Antifungal-active fractions were developed on a silica gel (60 F$_{254}$, Merck) TLC plate with butanol–acetic acid–water (4:1:2). After TLC plates were air-dried, silica gel bands with antifungal activity against *P. capsici* and *M. grisea* were observed under UV light or the anisaldehyde–sulfuric acid color reaction. Bands were collected and extracted with methanol.

The active eluates were developed by TLC using the bioautographic technique (Homans and Fuchs, 1970; Lazarovits et al., 1982). Active residues were loaded as a spot on a 3 × 8 cm silica gel plate (60 F$_{254}$, 0.2 mm thickness, Merck) and developed with chloroform–methanol (9:1) for SW-A and SW-B and with butanol–acetic acid–water (4:1:2) for SW-C, SW-D, and SW-E. TLC plates were allowed to air-dry. Twenty milliliters of 2% water agar was poured into a 15 cm diameter Petri dish. One chromatographed TLC plate was placed onto the surface of the solid agar, after the water agar was solidified completely. Molten V8 juice agar containing zoospore suspension of 1 × 10^5 zoospores mL^{-1} was spread uniformly onto TLC plates. After incubation for 3 days at 28 °C, plates were stained with 0.1% naphthol blue black solution in 5% acetic acid for 2–3 min. Plates were destained with 5% acetic acid for 1 h on a reciprocal shaker. The inhibition zones produced by each active residue were visualized using the bioautographic technique. The residues were further purified by preparative TLC on a silica gel (60 GF$_{254}$, Merck) plate (20 × 20 × 0.2 cm). Antibiotic-active zones were visualized under UV light, scraped, extracted with methanol for 24 h, and filtered through Whatman No. 5 paper. Filtered antibiotic eluates were concentrated *in vacuo*. To ascertain these TLC eluates, analytical HPLC was performed using a Waters 500A system (column: LiChrosorb RP-18, 5 μm, 4 × 250 mm, Merck). The antibiotic SW-A was eluted with acetonitrile–methanol–water (60:20:20) at a flow rate of 0.5 mL min^{-1} and detected at an absorbance of 230 nm. The antibiotics SW-B and SW-C were eluted with methanol–water (80:20) at 0.5 mL min^{-1} flow rate and detected at absorbances of 230 and 240 nm, respectively. The antibiotic SW-D was eluted with methanol–water (50:50) at a flow rate of 0.5 mL min^{-1} and detected at 240 nm. Antibiotics from each active region were determined by analytical HPLC. Semi-preparative HPLC (column: LiChrosorb RP-18, 7 μm, 10 × 250 mm, Merck) was then performed to further purify the antibiotic SW-B.

Structure Elucidation of the Antibiotic SW-B. Nuclear magnetic resonance (NMR) spectra of the purified antibiotic SW-B were recorded on a Bruker AMX 500 NMR spectrometer. ^{1}H- and ^{13}C-NMR spectra were measured in CD$_3$OD at 125.77 MHz. Chemical shifts of the two spectra are expressed in δ values (ppm). SW-B: EIMS, *m/z* 209 (M$^+$, 16), 165 (21), 124 (100), 111(46), 109 (43); HREIMS, *m/z* 209.1765 (M$^+$, C$_{13}$H$_{23}$-NO requires 209.1780); UV (MeOH) λ$_{max}$ 208, 260 nm; ^{1}H NMR (CD$_3$OD, 500 MHz) δ 0.89 (3 H, t, *J* = 7.1 Hz, H-10), 0.98 (3

H, d, *J* = 6.7 Hz, 6-Me), 1.25–1.30 (2 H, m, H-7), 1.28–1.35 (2 H, m, H-9), 1.35–1.40 (2 H, m, H-8), 1.81 (3 H, d, *J* = 1.2 Hz, 4-Me), 1.97 (3 H, d, *J* = 1.4 Hz, 2-Me), 2.49 (1 H, m, H-6), 5.27 (1 H, d, *J* = 9.7 Hz, H-5), 6.77 (1 H, s, H-3); ^{13}C NMR (CD$_3$OD, 125 MHz) δ 175.4 (C-1), 142.0 (C-5), 140.2 (C-3), 131.7 (C-4), 129.4 (C-9), 38.3 (C-7), 33.9 (C-6), 31.0 (C-8), 23.9 (C-9), 21.2 (6-Me), 16.7 (4-Me), 14.5, 14.4. ^{1}H–^{1}H correlation spectroscopy (COSY) and nuclear Overhauser effect (NOE) spectroscopy (NOESY) were performed on a Bruker AMX 600 NMR spectrometer. High- and low-resolution mass spectra (MS) were recorded on a JEOL HX 100A-HX 100A tandem mass spectrometer using the electron impact (EI) method. The UV absorption spectrum was measured with a Unikon 930 spectrometer (Kontron instrument).

Antifungal and Antibacterial Bioassays. Bioassays were conducted for evaluating antifungal and antibacterial activities. A portion (milligram) of the yellow-brown oily powder (120 g) from methanol extracts of hyphal mats was dissolved in a given volume of distilled water such that the final concentration was 1000 μg mL^{-1}. This was serially diluted to give concentrations of 1, 10, 100, and 1000 μg mL^{-1}. Disks (8 mm in diameter) inoculated with 16 plant pathogenic fungi were placed on potato dextrose agar containing crude antibiotic. The diameter of fungal mycelia was measured when control plates (8 cm in diameter) were completely covered with fungal mycelia, and the inhibition rate of mycelial growth at each concentration was calculated. Bioassay of SW-B for antifungal activity was done using hyphal growth inhibition assay (Broekaert et al., 1985) with six plant pathogenic fungi, which included *Alternaria mali, Cladosporium cucumerinum, Colletotrichum gloeosporioides, Fusarium oxysporum* f.sp. *cucumerinum, M. grisea,* and *P. capsici.* The 100 μL amounts of spore suspensions (10^6 spores mL^{-1}) in potato dextrose broth were incubated in sterilized microtubes. Spore suspensions of *C. cucumerinum* and other tested fungi were incubated at 22 and 28 °C, respectively, until the hyphae of the germlings had an average length of 30 μm. Then, solutions of the antibiotic SW-B at different concentrations were added to the germlings and the microtubes were reincubated at 22 or 28 °C until the control germlings attained an average length of 400 μm. The length of 60 individual hyphae was measured. Inhibition of relative hyphal growth is expressed as percentage of the hyphal growth of control cultures.

Antimicrobial activity of the antibiotic SW-B against bacteria and yeasts was examined using the antimicrobial bioassay method reported by Nair et al. (1992). The cell suspension cultures of the test organisms were prepared in 0.5 mL of nutrient broth to obtain a final concentration of 10^4 cfu mL^{-1}. Six bacteria, *Bacillus subtilis, Erwinia carotovora* subsp. *carotovora, Micrococcus luteus, Pseudomonas putida, Pseudomonas solanacearum,* and *Pseudomonas syringae* pv. *syringae,* and two yeasts, *Candida albicans* and *Saccharomyces cerevisiae,* were used as the test organisms. Solutions of the antibiotic SW-B at different concentrations were added into flat-bottom multiwell plates (Greiner) seeded with 10^4 cfu mL^{-1} of the test organisms. The inoculated plates were shaken and incubated at 28 °C. Growth of the test organisms was evaluated after a 2-day incubation period.

RESULTS AND DISCUSSION

Purification of Antifungal Compounds from Cultures of *S. flaveus* Strain A-11. The antibiotic SW-B, active against *P. capsici,* was purified from the culture filtrates of *S. flaveus* strain A-11 using silica gel column chromatography, Sephadex LH-20 column chromatography, preparative TLC, and finally HPLC.

Solid GYP agar cultures (24 kg) of *S. flaveus* strain A-11 were extracted with methanol (30 L). The culture filtrates were freeze-dried to yield yellow-brown oily powders (120 g). Antifungal activity of the crude antibiotics was evaluated using 16 plant pathogenic pathogenic fungi (Table 1). The crude antibiotic was most effective against *P. capsici* and *Sclerotinia sclero-*

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Table 1. Antifungal Activity of Culture Filtrates[a] of *S. flaveus* Strain A-11 to Various Plant Pathogenic Fungi

| test fungus	% inhibition of mycelial growth[b] by concentration of			
	1 μg/mL	10 μg/mL	100 μg/mL	1000 μg/mL
A. mali	2.5 ± 0.8	4.1 ± 0.8	6.5 ± 1.2	23.8 ± 0.9
A. solani	20.7 ± 1.8	29.1 ± 0.8	31.8 ± 1.7	59.0 ± 1.4
B. dothidea	4.3 ± 2.0	5.5 ± 2.5	30.0 ± 1.2	52.9 ± 0.3
C. capsici	22.0 ± 0.8	22.3 ± 0.8	43.5 ± 2.6	57.0 ± 1.4
Cercospora kikuchi	7.3 ± 0.8	9.0 ± 0.8	26.0 ± 1.2	42.2 ± 1.2
C. cucumerinum	11.2 ± 1.6	23.1 ± 0.8	38.0 ± 1.2	49.2 ± 1.3
C. gloeosporioides	1.5 ± 0.4	3.1 ± 2.4	4.4 ± 1.4	4.9 ± 1.4
Cylindrocarpon destructans	2.5 ± 0.4	3.4 ± 1.3	10.1 ± 1.4	45.8 ± 0.4
F. oxysporum f.sp. *cucumerinum*	2.0 ± 1.4	4.0 ± 0.4	7.2 ± 1.2	15.3 ± 1.4
M. grisea	1.7 ± 0.9	2.1 ± 0.4	7.4 ± 1.4	51.2 ± 0.4
Mycosphaerella fragariae	5.9 ± 2.4	9.5 ± 3.3	13.2 ± 0.9	22.7 ± 0.9
Mycosphaerella melonis	0.9 ± 0.8	14.8 ± 0.4	20.3 ± 1.2	24.4 ± 1.4
P. capsici	9.4 ± 1.6	11.9 ± 0.4	19.4 ± 1.2	67.9 ± 0.9
Pythium ultimum	0.0 ± 0.0	3.4 ± 0.4	10.1 ± 0.4	45.8 ± 1.5
R. solani	6.7 ± 0.9	16.4 ± 1.4	17.3 ± 1.2	57.5 ± 0.9
S. sclerotiorum	3.8 ± 0.9	28.7 ± 0.9	62.0 ± 1.2	70.9 ± 1.2

[a] Fungal mycelial growth was measured on potato dextrose agar containing different amounts of freeze-dried culture filtrates of *S. flaveus* strain A-11 when control plates (8 cm indiameter) were completely covered with fungal mycelia. [b] % inhibition of mycelial growth = [1 − (diameter of mycelial growth on antibiotic-treated plate/diameter of mycelial growth on untreated plate)] × 100. Each value represents a mean ± standard deviation of three replications.

tiorum among the fungi tested. In particular, mycelial growth of *S. sclerotiorum* was inhibited more than 50% at 100 μg mL^{-1}. Antifungal activity was also detected against *Alternaria solani, Botryosphaeria dothidea, Cercospora capsici, M. grisea*, and *Rhizoctonia solani,* whereas *C. gloeosporioides* and *F. oxysporum* f.sp. *cucumerinum* showed little inhibition even at 1000 μg mL^{-1}.

Freeze-dried culture filtrates (120 g) of *S. flaveus* strain A-11 were chromatographed on silical gel column with stepwise gradients of butanol−methanol. Each eluate of butanol−methanol was concentrated to a small volume and bioassayed against *P. capsici* and *M. grisea* using the paper disk method. Eluates of butanol−methnol (90:10, 60:40) were highly active against *P. capsici* and *M. grisea*.

The silica gel column eluates (butanol−methanol = 90:10, 60:40) that showed strong activity against *P. capsici* and *M. grisea* were further chromatogaphed on a Sephadex LH-20 column. Among the 500 fractions obtained from Sephadex LH-20 column chromatography of the silica gel column eluates (butanol−methanol = 90:10), fractions 61−76 (SW-A), 81−116 (SW-B), 121−156 (SW-C), and 161−176 (SW-D) showed higher activity against *P. capsici* and *M. grisea*. However, fractions 116−146 (SW-E) were inhibitory to both fungi.

In bioautography using TLC plates, SW-A and SW-B (developed by chloroform−methanol = 90:10 v/v) had antibiotic activity against *P. capsici* in the position of R_f 0.5−0.6, while SW-C, SW-D, and SW-E (developed by butanol−acetic acid:water = 40:10:20) were active in R_f 0.8. Methanol eluates of the bands that showed antifungal activity on preparative TLC plates were further purified by semipreparative HPLC. Pure antibiotics active against *P. capsici* were obtained from a single peak of the active region. Eluates of HPLC were concentrated and dissolved in a small volume of 100%
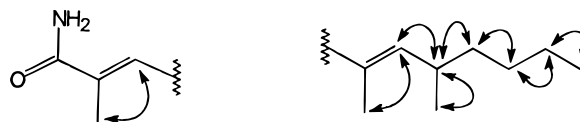

Figure 1. Partial structures of the antibiotic SW-B from ^{1}H−^{1}H COSY spectrum.
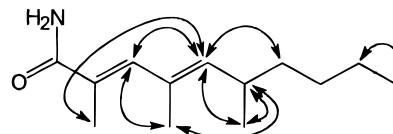
Figure 2. Correlations of the antibiotic SW-B from the NOESY spectrum.

methanol and then evaporated using nitrogen gas. Pale yellow crystal antibiotic SW-B (3 mg) resulted. HPLC analysis of SW-B gave a single peak with a retention time of 22.18 min at 230 nm.

Following silica gel column chromatography, most antifungal substances active against *P. capsici* and *M. grisea* were detected in eluates of butanol−methanol (90:10 and 60:40). However, eluates of butanol−methanol (40:60, 10:90, and 0:100) rarely contained antifungal substances. Five different antifungal substances were obtained from Sephadex LH-20 column chromatography with eluates of butanol−methanol (90:10 and 60:40). Of the five, four antifungal substances (SW-A, SW-B, SW-C, and SW-E) inhibited more effectively the mycelial growth of *P. capsici* than *M. grisea in vitro*. The methanol eluates of the bands that showed antifungal activity on the preparative TLC plates were concentrated, followed by further purification using semipreparative HPLC. This resulted in four pure antibiotics (SW-A, SW-B, SW-C, and SW-D).

Structure Elucidation of the Antibiotic SW-B. Structures of the antibiotic SW-B were elucidated by NMR and mass spectral analyses. The electron impact (EI) mass spectrum of SW-B confirmed its molecular weight to be 209: EI-MS 209 (M+, 16%), 165 (21%), 124 (100%), 111 (46%), and 109 (43%). The molecular formula of SW-B was deduced as $C_{13}H_{23}NO$ (calculated 209.1780) on the basis of the analysis of high-resolution EI-mass at m/z 209.1756 (M+). The formula was confirmed from proton and carbon counts by the spectral analysis of ^{1}H NMR and ^{13}C NMR. The ^{1}H-NMR spectrum of SW-B indicated the presence of four methyl groups at δ 0.89, 0.98, 1.81, and 1.97. The ^{1}H−^{1}H COSY spectrum of SW-B indicated that the two methyl groups (δ 1.97, J = 1.4 Hz; δ 1.81, J = 1.1 Hz) coupled with the two olefinic protons (δ 6.77 and 5.27). The remaining two methyl groups at δ 0.98 and 0.89 were coupled with the methine proton at δ 2.49 and with the methylene protons at δ 1.28−1.35, respectively. The methine proton was coupled with the olefinic proton at δ 5.27 and the methylene protons at δ 1.25−1.30. The molecular formula suggests that the partial structures of SW-B (Figure 1) deduced from the ^{1}H−^{1}H COSY spectral analysis may be connected to each other, indicating a diene moiety. The possible connectivity between two partial structures was confirmed from correlations in the NOESY spectrum: H-3 to H-5 and 4-CH$_3$; H-5 to 2-CH$_3$ (Figure 2). The antibiotic SW-B exhibited UV absorption maxima at 260 nm in methanol. The configuration of the two alkenyl double bonds was determined to be entgegen, *E*, since 3-H and 5-H showed no NOE with 2-CH$_3$ and 4-CH$_3$ groups, respectively. NOE
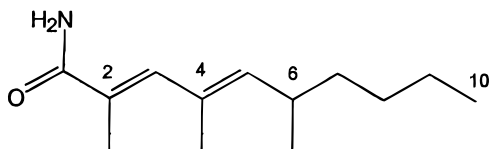
**Figure 3.** Structure of the antibiotic SW-B, 2,4,6-trimethyl-deca-(2*E*,4*E*)-dienamide.

correlation patterns are shown in Figure 2. In light of all the spectral data, the structure of the antibiotic SW-B was determined to be 2,4,6-trimethyldeca-(2*E*,4*E*)-dienamide (molecular formula $C_{13}H_{23}NO$) (Figure 3). The molecular weight of SW-B was 209.178. From the proposed structure of SW-B (Figure 3), the antibiotic SW-B was concluded to be a member of the manumycin group of antibiotics (Schröder and Zeeck, 1973; Zeeck et al., 1987, 1993).

Members of the manumycin group of antibiotics contain a multifunctional mC_7N unit as a central structural element. This moiety, consisting of a six-membered carbocyclic ring bearing one carbon and one nitrogen atom in a meta position, is quite common in nature (Hornemann et al., 1974). Some manumycin group antibiotics such as asukamycin (Omura et al., 1976) and nisamycin (Hayashi et al., 1994a,b) have been found since the initial discovery of manumycin by Buzzetti et al. (1963). Manumycin is active against Gram-positive bacteria, fungi, and L-1210 leukemia stem cells, but shows no activity against Gram-negative bacteria or yeasts (Zeeck et al., 1987). In addition, manumycin exhibits an inhibitory activity against polymorphonuclear leukocyte elastase.

The antibiotic SW-B as a derivative of manumycin turned out to have the same structure as the manumycin-type compound 64p-A which has been produced from *Streptomyces parvulus* by precursor-directed biosynthesis (Zeeck et al., 1993; Kaiser et al., 1994). The nonchiral 64p-A is the carboxamide of a shortened polyketide chain, which represents the branched side chain of the manumycin molecule.

The antifungal activity of the pure SW-B against six plant pathogenic fungi was evaluated using hyphal growth inhibition assay (Figure 4). SW-B showed a high level of inhibitory activity against *P. capsici, M. grisea, C. cucumerinum,* and *A. mali.* Hyphal growth of *P. capsici, C. cucumerinum,* and *M. grisea* was inhibited by more than 50% at 10 μg mL^{-1} and by about 90% at 50 μg mL^{-1}. However, *F. oxysporum* f.sp. *cucumerinum* and *C. gloeosporioides* were relatively insensitive to the SW-B, even at a concentration of 50 μg mL^{-1}. No antimicrobial activity of SW-B against *B. subtilis, E. carotovora* subsp. *carotovora, M. luteus, P. putida, Ps. solanacearum, Ps. syringae* pv. *syringae, C. albicans,* and *S. cerevisiae* was observed (no data presented). Since SW-B represents a structural part of manumycin, the possibility of using SW-B as a core compound to develop agricultural fungicides is promising.

The production of SW-B by *S. flaveus* in the glycerol yeast extract peptone agar may be due to the incorporation of glycerol into SW-B molecule during its biosynthesis. Recently, Kaiser et al. (1994) demonstrated that manumycin production could be enhanced by feeding glycerol and by increasing the dissolved oxygen content. Further studies will be necessary to determine important parameters for mass production of manumycin-type metabolites in a culture of *S. flaveus* strain A-11.
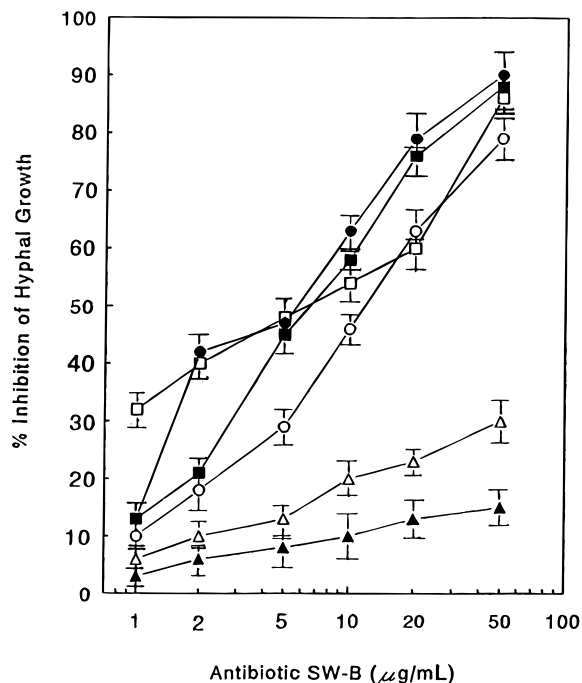
Figure 4. Inhibitory activity of the antibiotic SW-B against different plant pathogenic fungi: (○) *A. mali;* (□) *M. grisea;* (△) *F. oxysporum* f.sp. *cucumerinum;* (●) *C. cucumerinum;* (■) *P. capsici;* (▲) *C. gloeosporioides.* Inhibition of hyphal growth by treatment with SW-B is expressed as percentage of the hyphal growth of control cultures. Data are means of six independent experiments. Each bar represents a standard deviation.

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