Antifungal Activity of Oosporein from an Antagonistic Fungus against *Phytophthora infestans*

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An antifungal metabolite, oosporein, was isolated from the culture of *Verticillium psalliotae* that produced the antagonistic effects on *Phytophthora infestans*. Oosporein exhibited a significant growth-inhibitory effect on *P. infestans* in comparison with other phytopathogenic fungi.

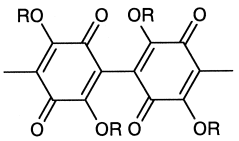
Key words: Oosporein, Antifungal Activity, *Phytophthora infestans*

Introduction

Late blight caused by *Phytophthora infestans* is a serious disease in solanaceous crops. To investigate biological control of late blight of tomato (*Lycopersicon esculentum* Mill.), a fungus, *Verticillium psalliotae*, was selected as an antagonist against *P. infestans* by screening of fungal isolates from several animal-waste composts in our laboratories. The culture filtrate of the antagonist exhibited antifungal activity against *P. infestans*. Although it was reported that *V. psalliotae* produced a bioactive compound such as oosporein (**1**) (Wainwright and Betts, 1986), the antibiotic effects of this fungus and this compound on *P. infestans* were not known. This report describes the isolation of a known compound, oosporein (**1**) from the culture of *V. psalliotae* and its antifungal activity against *P. infestans* and also other phytopathogenic fungi.

Results and Discussion

The growth of *P. infestans* was completely inhibited on the media containing the culture filtrate of *V. psalliotae* in bioassay. The culture filtrate of *V. psalliotae* was extracted with ethyl acetate under acidic condition, and then compound **1** was isolated from the ethyl acetate extracts as a acetonitrile-insoluble substance. Compound **1** was methylated with diazomethane to yield the tetramethyl ether (**1a**). The structure of **1** was elucidated as oosporein (Cole *et al.*, 1974) from the NMR, MS and UV spectral data of **1** and **1a** (Fig. 1). Oosporein was also isolated as a toxin to plants and poultry from the culture of *Beauveria* and *Chaetomium* species (Vining *et al.*, 1962; Cole *et al.*, 1974; Manning and Wyatt, 1984). The risk assessments of oosporein were reviewed in detail, because one of the oosporein-producers, *B. brongniartii*, is an important entomopathogenic fungus utilized as a biological control agent for pest control (Strasser *et al.*, 2000).



1 R = H
1a R = Me

Fig. 1. Structures of oosporein (**1**) and oosporein tetramethyl ether (**1a**).

The inhibitory effects of oosporein were evaluated on radial growth of *P. infestans* and other tomato pathogenic fungi, *Alternaria solani* and *Fusarium oxysporum* (Table I). Oosporein exhibited a strong inhibitory activity especially against *P. infestans* (MIC 16 μM). *A. solani* and *F. oxysporum* were, however, insensitive. Wainwright *et al.* (1986) described that oosporein showed weak antibacterial activities, and no significant effects on six species of fungi. The antifungal activities of oosporein against several species were found in acidic, but not in neutral solution (Taniguchi *et al.*, 1984). In this report we suggest that oosporein would be a selective antibiotic to *P. infestans*.

Experimental

General experimental procedures

NMR: JEOL GSX-500 instrument, 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR) in CD_3OD, or $CDCl_3$. MS: APCI on Finnigan-MAT LCQ mass spectrometer by direct injection using a syringe pump. UV: Hitachi U-3310 spectrophotometer. Silica gel 60 F_{254} TLC plates (thickness

Table I. Inhibitory effects of **1** on growth of tomato pathogenic fungi.

Pathogen	Growth (% of control)							MIC	
	Concentration [μg/ml]							[μg/ml]	[μM]
	1	2	5	10	20	50	100		
Phytophthora infestans	99	97	0	0	0	–	–	5	16
Alternaria solani	–	–	–	112	98	57	32	> 100	> 327
Fusarium oxysporum	–	–	–	98	94	92	73	> 100	> 327

–: Not tested.

0.25 mm; Merck, Darmstadt, Germany) were used for analytical and preparative TLC. CC was carried out on silica gel 60 (Merck, Darmstadt, Germany).

Cultivation of antagonistic fungi

An isolate of antagonistic fungus from an animal-waste compost, *Verticillium psalliotae* (MAFF 239144), was grown on potato dextrose agar (PDA) slants to use in the experiments. The fungus was inoculated into three Erlenmeyer flasks (500 ml) containing 200 ml of potato dextrose broth (PDB) and incubated in darkness for 14 d at 25 °C without shaking. Fungal biomass yield was 4.9 mg dry matter/ml.

Isolation of antifungal metabolite

The culture of *V. psalliotae* (600 ml) was filtered and evaporated to 200 ml *in vacuo*. The solution was extracted three times with 200 ml of EtOAc after adjusting the pH to 2.0 with 2 M HCl. The EtOAc layer dried over Na_2SO_4 was concentrated to dryness under reduced pressure. The EtOAc extracts (110 mg) were suspended in a small volume of MeCN, and compound **1** (75 mg) was obtained as insoluble residues by filtration. Methylation of compound **1** (15 mg) with CH_2N_2 gave the tetramethyl ether (**1a**, 7 mg).

Oosporein (**1**): Bronze powder. – APCIMS (positive ions): *m/z* (rel. int.) = 307 (75) $[M+H]^+$, 289 (28), 279 (100). – APCIMS (negative ions): *m/z* (rel. int.) = 306 (100) $[M]^-$, 289 (2), 278 (21). – ^{1}H NMR (500 MHz, CD_3OD): δ = 1.90 (6H, s). – ^{13}C NMR (125 MHz, CD_3OD): δ = 8.5 (2C), 108.9 (2C), 115.1 (2C). – UV (MeOH): λ_{max} (ε) = 289 nm (34,300).

Oosporein tetramethyl ether (**1a**): Orange powder. – APCIMS (positive ions): *m/z* (rel. int.) = 363 (100) $[M+H]^+$, 348 (11). – APCIMS (negative ions): *m/z* (rel. int.) = 362 (100) $[M]^-$, 347 (64). – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.96 (6H, s), 3.99 (6H, s), 4.03 (6H, s). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 8.6 (2C), 60.9 (2C), 61.1 (2C), 117.8 (2C), 126.6 (2C), 155.8 (2C), 156.3 (2C), 181.4 (2C), 183.6 (2C).

Bioassay

Three pathogenic fungi against tomato were used for the bioassay: *Phytophthora infestans* (MAFF 239145), *Alternaria solani* (MAFF 239146) and *Fusarium oxysporum* (MAFF 239147).

Antifungal activity was examined according to the method described previously (Nagaoka *et al.*, 2001). Each culture of fungi was filtered with a membrane filter (pore size 0.45 μm), and then 1 ml of filtrate was mixed with 9 ml of rye A agar medium (Caten and Jinks, 1967; Hartman and Huang, 1995) in a test tube and transferred to a Petri dish (9 cm in diameter). Mycelial plugs of *P. infestans* were placed in the center of plates, and the inoculated plates were incubated in the dark at 15 °C for 10 d. The extracts and fractions from fungal cultures were dissolved in distilled water or 10% MeOH at appropriate concentrations, and applied to the bioassay in the same way.

Compound **1** was dissolved in a small volume of 0.01 M NH_4OH and quickly evaporated to dryness, and re-dissolved in distilled water at 0.1, 0.2, 0.5, 1, 2, 5 and 10 mg/ml (corresponding to 0.3, 0.7, 1.6, 3.3, 6.5, 16.3 and 32.7 mM) (pH 6.5). Each solution (100 μl) was mixed with 9.9 ml of rye A agar medium for the bioassay on the growth of *P. infestans* as described above. Antifungal activities of compound **1** against other phytopathogenic fungi, *A. solani* and *F. oxysporum*, were tested in the similar way, except that each solution (150 μl) was mixed with 14.85 ml of potato dextrose agar (PDA) medium. The inoculated plates were incubated at

25 °C for 6 d in the case of *A. solani* and at 25 °C for 10 d in the case of *F. oxysporum*. All the procedures were duplicated. After the incubation radial growth on the media containing the sample was divided by the growth of control to provide the percentages of growth. Minimum inhibitory concentrations (MICs) which completely inhibited fungal growth were also indicated.

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Caten C. E. and Jinks J. L. (1967), Spontaneous variability of single isolates of *Phytophthora infestans*. I. Cultural variation. Can. J. Bot. **46**, 329–348.

Cole R. J., Kirksey J. W., Cutler H. G., and Davis E. E. (1974), Toxic effects of oosporein from *Chaetomium trilatelare*. J. Agr. Food Chem. **22**, 517–522.

Hartman G. L. and Huang Y. H. (1995), Characteristics of *Phytophthora infestans* isolates and development of late blight on tomato in Taiwan. Plant Dis. **79**, 849–852.

Manning R. O. and Wyatt R. D. (1984), Comparative toxicity of *Chaetomium* contaminated corn and various chemical forms of oosporein in broiler chicks. Poultry Sci. **63**, 251–259.

Nagaoka T., Goto K., Watanabe A., Sakata Y., and Yoshihara T. (2001), Sesquiterpenoids in root exudates of *Solanum aethiopicum*. Z. Naturforsch. **56c**, 707–713.

Strasser H., Vey A., and Butt T. M. (2000), Are there any risks in using entomopathogenic fungi for pest control, with particular reference to the bioactive metabolites of *Metarhizobium*, *Tolypocladium* and *Beauveria* species? Biocontrol Sci. Technol. **10**, 717–735.

Taniguchi M., Kawaguchi T., Tanaka T., and Oi S. (1984), Antimicrobial and respiration-inhibitory activities of oosporein. Agr. Biol. Chem. **48**, 1065–1067.

Vining L. C., Kelleher W. J., and Schwarting A. E. (1962), Oosporein production by a strain of *Beauveria bassiana* originally identified as *Amanita muscaria*. Can. J. Microbiol. **8**, 931–933.

Wainwright M. and Betts R. P. (1986), Antibiotic activity of oosporein from *Verticillium psalliotae*. Trans. Br. Mycol. Soc. **86**, 168–170.