Spherical Swelling of Germ Tubes and Hyphae of *Fusarium oxysporum* by the Treatment with 5-fluoroindole

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In our attempts to develop effective chemical control agents for soil-borne phytopathogenic bacteria of tomato, we found that indole and its related compounds (3-indolepropionic acid and 3-indoleacrylic acid) were capable of inhibiting the multiplication of *Pseudomonas solanacearum*^{3,4,8)}, the pathogen causing the bacterial wilt of tomato, and have clarified the correlation between chemical structures of these compounds and their antibacterial activities^{5,6)}. These compounds were examined for their antifungal spectra against a variety of tomato pathogens, *Cladosporium fulvum*, *Botrytis cinerea*, *Erysiphe cichoracearum*, *Fusarium oxysporum* f. sp. *lycopersici*, *Rhizoctonia solani*, *Pythium aphanidermatum*, *Verticillium albo-atrum* and *Phytophthora infestans*, and were shown to be effective only to *E. cichoracearum*. Recently, 5-fluoroindole (5-FI) was found to inhibit the growth of some soil-borne pathogens, such as *F. oxysporum*, *R. solani* and *P. aphanidermatum*. In the present study, we report the spherical swelling of germ-tubes and vegetative hyphae of *F. oxysporum* f. sp. *lycopersici* by the treatment with 5-FI and discuss the antifungal activity of this compound.

Screening of antifungal compounds. Microconidia of *F. oxysporum* Schlechtendahl f. sp. *lycopersici* (Saccardo) Snyder et Hansen, race I (KFOL-01) were collected from 10-day-cultures of an agar-solidified Czapek medium⁷⁾ and spread onto the same solid medium. After 4 days of incubation at 26°C in dark, the edge portion of mycelial colonies was clipped by a cork borer (4 mm in diameter) and transferred to a solid Czapek medium containing 100 µg/ml of test compounds. The test medium was prepared by dissolving the compounds in 1% methanol and mixing them with an equal volume of a melted 2×Czapek-agar medium. This concentration of organic solvent (0.5% methanol) was shown to be non-toxic to the fungal growth in our preliminary work. The compounds used were purchased from Sigma Chemical Co., St. Louis, MO, USA. After 6

days of incubation, the antifungal activity of the compounds was determined by measuring the radial growth of mycelia from discs. In the present screening of the antifungal compounds, we examined 145 indole related compounds (data not shown) and eventually found that 5-FI strongly inhibited the mycelial growth of KFOL-01.

Assay for effect on germination, mycelial growth, and sporulation. The density of microconidia was adjusted to 10⁴ spores/ml in sterilized distilled water, and the conidial suspension was mixed with 9 parts of a melted Czapek-agar medium and thinly spread onto a glass slide. After solidification at room temperature, the conidia-embedded agar plates (fungal plate, 15×20×1 mm) were dipped into the test medium (liquid Czapek medium containing various concentrations of 5-FI) immediately or after some periods of incubation. Morphological change of the fungal structures was examined with an Olympus differential interference contrast Nomarski microscope. First we treated non-germinated microconidia of KFOL-01 with 5-FI and examined whether this compound could suppress the germination of the fungus. Figure 1 shows the germination rates of microconidia of KFOL-01 versus concentrations of the treated compound. The result indicated that 5-FI suppressed the conidial germination as an increase of concentrations of the compounds treated and that the treatment with 5-FI caused the spherical swelling of tips of germ tubes. As shown in Fig. 2, some of the microconidia initiated germination and produced germ tubes (Fig. 2A), but the tubes were swollen to form the spherical bodies (Fig. 2B). These spherical bodies increased their sizes and reached the maximum (approximately 15 µm in diameter) 6 hr after the treatment. The rates of spherical body formation were closely dependent on the concentrations of 5-FI and conspicuously high in microconidia treated with more than 25 µg/ml of 5-FI (Fig. 1). Either the elongation of germ tubes or the development of vegetative hyphae from the spherical

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Fig. 1. Germination of microconidia of *F. oxysporum* f. sp. *lycopersici* treated with 5-fluoroindole (5-FI). Microconidia were embedded in an agar-solidified medium and dipped into a test solution containing various concentrations of 5-FI for 15 hr. The rates of germination (open column) were expressed relative to the rate ($67.5 \pm 3.9\%$) of non-treated control. The shaded column represents the microconidia which formed spherical bodies on germ tubes. Data were given as means of five replications.

Fig. 2. Spherical swelling of germ-tube of microconidium of *F. oxysporum* f. sp. *lycopersici* by the treatment with 5-fluoroindole ($50 \mu\text{g/ml}$). The germ tube (arrow in A) swelled spherically and the spherical body reached the maximum diameter 6 hr after the treatment (B). Bar represents $10 \mu\text{m}$.

Fig. 3. Suppression of conidial formation of *F. oxysporum* f. sp. *lycopersici* by the treatment with 5-fluoroindole. The fungal plates were incubated for 72 hr and treated with $50 \mu\text{g/ml}$ of 5-fluoroindole. Vigorous formation of microconidia was observed in non-treated control (A). Note that the conidial formation was completely inhibited by the formation of spherical bodies in the pathogen (B) treated with 5-fluoroindole. Bar represents $20 \mu\text{m}$.

bodies was not observed in these microconidia.

The subsequent treatment with 5-FI was conducted using microconidia which had preliminarily cultured for various periods of times. Figure 3A shows the elongated vegetative hyphae forming the microconidia at the numerous sites in non-treated control. In the 5-FI-treated pathogen, however, the formation of conidia was completely inhibited and spherical bodies were formed at the tips and lateral sites of vegetative hyphae (Fig. 3B).

In order to clarify the correlation of biological activity of 5-FI with its chemical structure, we examined the effects of some substituents of 5-FI on the hyphal swelling and inhibition of mycelial growth of KFOL-01. As a result, the data indicated that the swelling was not induced either when fluorine of 5-FI was substituted by other atoms or radicals or when fluorine was transferred to other carbons of indole structure, although these

Table 1. Effects of indole derivatives on the formation of spherical bodies (SB) and the mycelial growth of *F. oxysporum* f. sp. *lycopersici*, race I.

Compounds	Formation of SB ^{a)}	Rate (%) of mycelial growth ^{b)}					
		1	5	10	25	50	100 ^{c)}
5-fluoroindole	+	100	86.1	78.8	36.1	0	0
5-bromoindole	—	100	96.9	75.0	34.4	0	0
5-chloroindole	—	97.1	92.1	84.2	34.2	18.4	0
5-hydroxyindole	—	89.4	89.4	97.0	97.0	90.9	83.3
5-methylindole	—	92.4	89.4	80.8	65.2	25.8	0
5-cyanoindole	—	84.8	87.9	87.9	83.3	77.3	42.4
indole-5-carboxylic acid	—	87.9	89.4	89.4	92.4	89.4	89.4
4-fluoroindole	—	100	100	100	87.5	0	0
6-fluoroindole	—	100	100	100	97.5	80.0	19.2
indole	—	100	100	100	100	100	70.8

a) Treatment with 100 $\mu\text{g/ml}$ of test compounds.

b) The growth rates were expressed relative to the rate of non-treated control. The data were given as means of five replications.

c) Concentrations ($\mu\text{g/ml}$) of test compounds. Fig. 4. Lysis of spherical body of germ tube of *F. oxysporum* f. sp. *lycopersici* by osmotic pressure. The spherical bodies were induced in the germ tubes of microconidia under the same condition as in Fig. 2 and subjected to running water. Photographs of spherical structure was continuously taken 4 (A), 8 (B), and 12 hr (C) after the treatment of osmotic shock. Bar represents 10 μm .

substituted compounds exhibited the inhibitory effect on the mycelial growth, depending on their concentrations (Table 1). These results indicate that the spherical swelling of hyphal tips could be specifically induced by the treatment with 5-FI, and the inhibition mechanism of

this compound seems to be different from an inhibitory activity of other substituted compounds.

Test for osmotic sensitivity of spherical bodies.

The fungal plate was placed on a glass slide, surrounded by a filter paper frame (5 mm wide) and sealed by a

cover glass. Distilled water was supplied to the fungal plate by continuously dropping water onto the paper frame with a glass capillary and flowed water was blotted with a dried filter paper at the other side. The lysis of the spherical bodies was observed under microscopy under the continuous flow of running water (Fig. 4). In addition, the osmotic stability of the bodies was examined by dipping the fungal plate in an aqueous solution containing various concentrations of an osmotic stabilizer (between 0.1 and 1.1 M mannitol) and the number of microconidia which maintained the spherical bodies was counted under microscopy. The result indicated that the spherical bodies were stably maintained by the addition of 0.7 M mannitol, suggesting that the spherical body induced by 5-FI was a protoplast-like structure sensitive to osmotic shock. Although the biological function of 5-FI in *F. oxysporum* has not been obvious, some workers have reported that the tryptophan biosynthetic pathway of homobasidiomycete, *Coprinus cinereus*⁹⁾ and the ectomycorrhizal basidiomycete *Hebeloma cylindrosporum*²⁾ was feedback-inhibited by 5-fluorotryptophan intracellularly converted from 5-FI. Bhattiprolu *et al.*¹⁾ conferred the resistance to 5-FI on *C. bilanatus* by integrating the mutant anthranilate synthetase gene (*trp3^{lar}*) from *C. cinereus*. The genetic complementation test by these mutant genes is underway in this fungus.

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和 文 摘 要

野々村照雄・豊田秀吉・反保宏行・松田克礼・松田一彦・Abdul GAFUR・道後充恵・大内成志：5-fluoroindole 処理によるトマト萎ちょう病菌の発芽管および菌糸先端の球状化

トマト萎ちょう病菌に対するインドール類縁化合物の増殖抑制効果を検討したところ、5-fluoroindole (5-FI) が顕著な抑制効果を示したので、この物質の作用についてさらに検討を加えた。その結果、5-FI を未発芽小型分生胞子に処理した場合には、50 $\mu\text{g/ml}$ の濃度でほぼ完全に発芽を抑制し、わずかに発芽したもののでも、発芽管の突起が形成された時点で球形に膨潤し、以後の伸長を停止した。また、あらかじめ培養して発芽管もしくは菌糸を伸長させた分生胞子に 5-FI を処理した場合にも、それらの先端部が球形に膨潤し、それ以後の伸長や胞子形成を抑制した。さらに、上記の球状構造体は浸透圧処理に感受性であることから、プロトプラスト様の構造であるものと推定された。

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