フォスフォリピッドとその関連物質が糸状菌のピサチン感受性に及ぼす影響

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The Effect of Phospholipids and Their Relative Components on the Pisatin Sensitivity of Several Fungi

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Summary

The effect of phospholipids and their relative components on the pisatin sensitivity of several fungi was studied. Phosphatidylcholine (400 μg/ml) decreased the pisatin sensitivity of *Ascochyta pisi, Fusarium solani* f. sp. *pisi, Glomerela cingulata* and *Cochliobolus miyabeanus* in spore germination test. Decreasing of the pisatin sensitivity of mycelial growth of *Pestalotia funerea* and *F. solani* f. sp. *pisi* was also observed with addition of phosphatidylcholine (400 μg/ml) and phosphatidylserine (1,000 μg/ml). Increasing of the concentration of phosphatidylcholine from 140 μg/ml to 400 μg/ml resulted in the increase of the effectivity of this lipid in reducing the pisatin sensitivity of the fungi tested. Addition of choline, serine, phosphocholine chloride calcium and total lipid to medium did not affect the pisatin sensitivity of the fungi. The findings in the present study illustrate the value of the lipids in the interaction of fungi and phytoalexin in laboratory and probably in the plants.

Key words: phospholipids, pisatin-sensitivity.

Introduction

In living system the structures of the cells and tissues are based on large molecules which consist of the proteins, polysaccharides and complex lipids[1]. Most of lipids are phospholipids[2]. The four major phospholipids found in all higher cells are phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine and spingomyeline[2]. In the healthy plants, phospholipids have been known to have very important roles not only as component of lipo-protein membrane but also specific function in plant cells such as in photosynthesis, fatty acid metabolism and in other cellular processes[3]. In addition, phospholipids have been reported to have ability to modify the sensitivity of fungi against phytoalexins[4, 5].

The purpose of these present studies was to examine the effect of addition of lipid, phospholipids (phosphatidylcholine and phosphatidylserine) and their relative components (serine, choline and phosphocholine chloride calcium) into culture media on the pisatin sensitivity of several fungi.

Materials and Methods

Fungi. Seven fungi (*Ascochyta pisi, Fusarium solani* f. sp. *pisi, Glomerella cingulata, Cochliobolus miyabeanus, Rhizopus stolonifer, Pestalotia funerea* and *Fusarium oxysporum* f. sp. *fragariae*) were isolated from their host plants and maintained on potato sucrose agar (PSA) at 25 C in the dark and transferred periodically to fresh medium in the laboratory.

Chemicals preparation. Phosphatidylcholine (Type IV-S, from soybean, 40% and 14%) and phosphatidylserine (97F-8425, 98%) were purchased from Sigma Chemical Company, ST. Louis, M.O; Choline chloride (99%) and phosphocholine chloride calcium (99%) from Katayama Chemical; DL-serine from Wako Pure Chemicals Industries. LTD. Total lipid was prepared by using the method of Foltch *et al*[6].

The spore germination bioassay. For spore germination bioassay, test agar block method was used[7]. Pisatin was added to the germination test water containing 2% ethanol (v/v) and the final concentrations of pisatin were 75 μg/ml and 125 μg/ml.

The mycelial growth bioassay. The method used in the bioassay was modification of the method of Pueppke and VanEtten[8]. The bioassay was performed in 30×10 mm petridishes containing 1 ml of sucrose peptone agar (SPA: 10 g of peptone, 5 g of sucrose, 20 g of agar, 1,000 ml of 0.04 M potassium phosphate buffer, pH 6.5). Pisatin extracted from *Pisum sativum* was added to the test media containing 2% ethanol (v/v) and the final concentration of pisatin was 75 μg/ml. SPA containing ethanol only was used as control. A 5 mm agar plug cut from mycelial colony grown on SPA was placed in the center of each bioassay plate medium. The transplanted plates were kept at 25 C in the dark. Fungal growth was determined periodically by measuring and averaging diameters of two perpendicular colony. Data are the mean of three experiments using two plates in each experiment.

Results

The assay with spore germination. In the preliminary test, it was found that the spores of *Ascochyta pisi, Fusarium solani* f. sp. *pisi, Glomerella cingulata* and *Cochliobolus miyabeanus* successfully germinated in the water agar, but *Rhizopus stolonifer* failed to germinate in the same medium. Thus the water agar was modified by the addition of sucrose (2%) and L-alanine (0.2%)[9]. In this modified medium germination rate of *R. stolonifer* was 82% while it was 0% in water agar medium. Both the germination rate and germ-tube length of spore were significantly increased by the addition of phosphatidylcholine to the modified medium, whereas it did not influence on the spore germination in the absence of pisatin (Table 1).

The assay with mycelial growth. Fig. 1 showed that the pisatin sensitivity of fungi reduced significantly by the addition of phosphatidylcholine and phosphatidylserine to medium. The pisatin sensitivity of fungi, on the other hand, could not be reduced by the addition of choline, serine, phosphocholine chloride calcium and total lipid to medium (Fig. 1).

Table 1 Effect of phosphatidylcholine on the pisatin sensitivity of five fungi using spore germination

Fungi	Treatment	Germination (%)	Average length of germ tube (μm)
Rhizopus stolonifer	Buffer	81.9	35.1
	PC*	80.7	37.6
	Pisatin (50 μg/ml)	0	0
	Pisatin+PC	14.5	17.5
Fusarium solani f. sp. *pisi*	Buffer	97.1	43.6
	PC	98.5	50.2
	Pisatin (75 μg/ml)	58.0	11.0
	Pisatin+PC	71.5	12.2
Ascochyta pisi	Buffer	90.5	17.5
	PC	95.0	20.3
	Pisatin (130 μg/ml)	45.5	7.7
	Pisatin+PC	57.2	10.5
Glomerella cingulata	Buffer	85.0	58.2
	PC	91.0	65.3
	Pisatin (130 μg/ml)	9.6	1.0
	Pisatin+PC	17.8	29.1
Cochliobolus miyabeanus	Buffer	82.0	48.5
	PC	88.0	55.3
	Pisatin (130 μg/ml)	6.6	1.9
	Pisatin+PC	12.2	23.3

* PC : Phosphatidylcholine
Data represent means of three replications in each experiment and 100 spores per replication were observed. Phosphatidylcholine was used at concentration of 400 μg/ml

Fig. 2 showed that serine did not influence the growth of fungi cultured on SPA with pisatin. The addition of phosphatidylcholine and choline to medium without pisatin did not influence the mycelial growth as shown in Fig. 3. Fig. 4 showed that the addition of large quantity (400 μg/ml) of phosphatidylcholine more reduced the pisatin sensitivity of fungi than that of small one (140 μg/ml).

Discussion

Phosphatidylcholine is the major phospholipids in most eukaryotic cell membranes, with phosphatidylethanolamine generally being second most abundant[10]. The ability of phospholipids to influence the effectivity of phytoalexins has been previously reported by Bull[4] with phaseollin and kievitone, and Sweigard and VanEtten[5] with pisatin. In this study, similar result was found that phosphatidylcholine and phosphatidylserine reduced the pisatin sensitivity in mycelial growth (Fig. 1, 4) and spore germination (Table 1) of the fungi. There is no information concerning the mechanism of reduction against phytoalexin sensitivity by phospholipids so far. It is suggested that phospholipids was used to alter cell membrane damage caused by pisatin[5]. In addition it was also possible that phospholipids increased the activity of ATPase[11] which is needed for the fungi to overcome the pisatin.

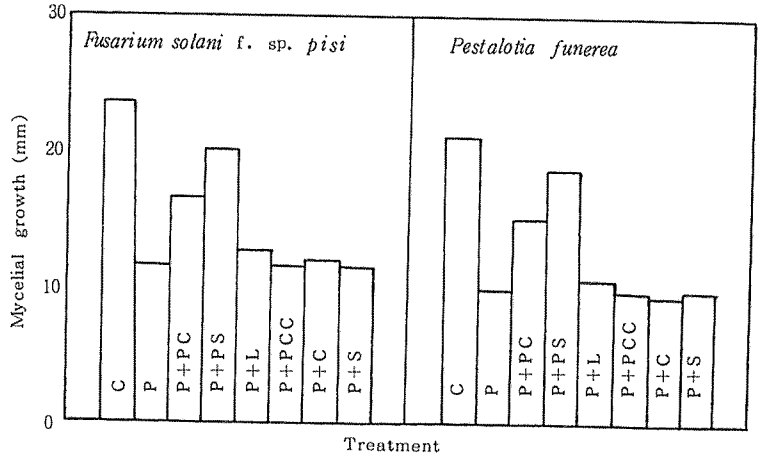

Fig. 1 Effect of phospholipids and their relative components on the pisatin sensitivity of two fungi.
C : Control P : pisatin (75 μg/ml)
P+PC : pisatin+phosphatidylcholine
P+PS : pisatin+phosphatidylserine
P+L : pisatin+total lipid
P+PCC : pisatin+phosphocholine chloride calsium
P+C : pisatin+choline
P+S : pisatin+serine
PC, PS, L, PCC, C and S were used at concentration of 400 μg/ml; 980 μg/ml; 1,000 μg/ml; 990 μg/ml; 990 μg/ml; and 990 μg/ml; respectively

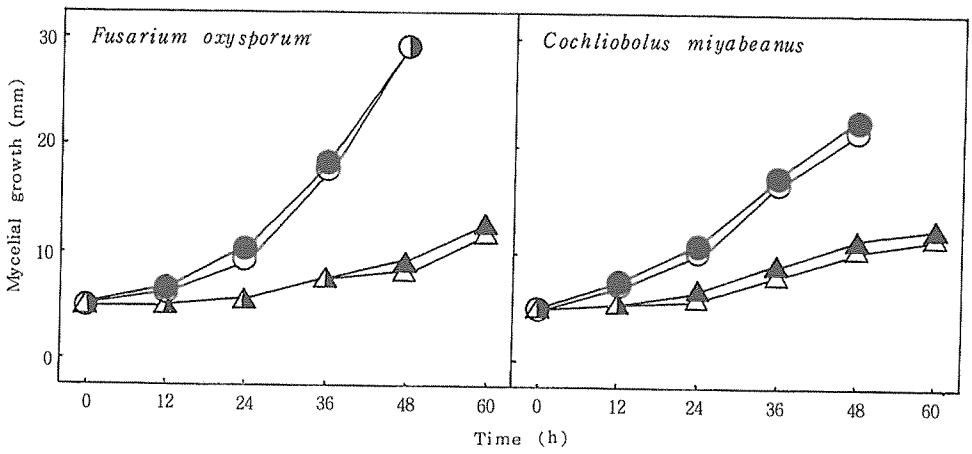

Fig. 2 Mycelial growth of two fungi on sucrose peptone agar without (○) and with serine (●), pisatin (△), pisatin+serine (▲). Serine was added at the final concentration of 1,000 μg/ml.

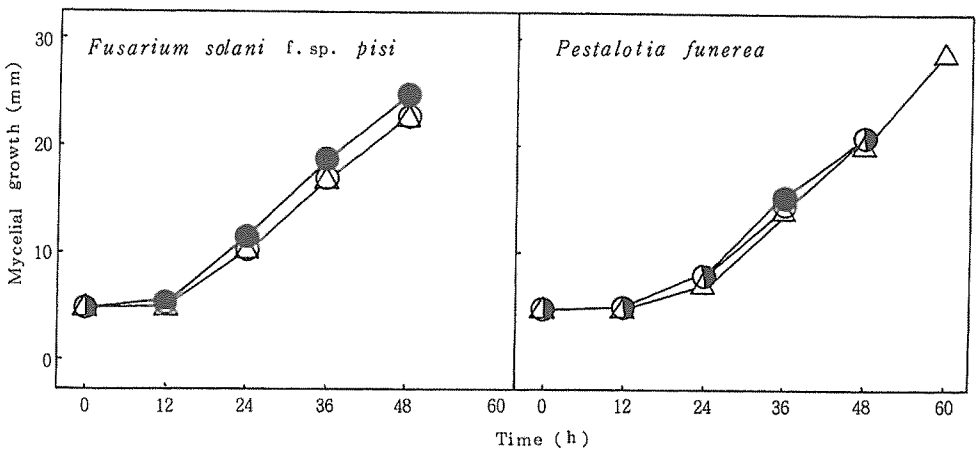

Fig. 3 Mycelial growth of two fungi on sucrose peptone agar without (○) and with phosphatidylcholine (●), choline (△). Phosphatidylcholine were added at the final concentration of 400 μg/ml and 1,000 μg/ml, respectively.

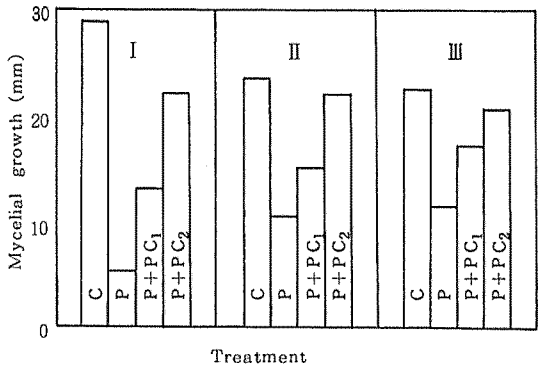

Fig. 4 Effect of different concentrations of phosphatidylcholine on the pisatin sensitivity of three fungi.
I : *Rhizopus stolonifer*
II : *Cochliobolus miyabeanus*
III : *Glomerella cingulata*
C : Control P : pisatin (75 μg/ml)
$P+PC_1$: pisatin+phosphatidylcholine (140 μg/ml)
$P+PC_2$: pisatin+phosphatidylcholine (400 μg/ml)

Addition of the relative components of phospholipids such as choline, serine and phosphocholine chloride calcium failed to inhibit pisatin activity (Fig. 1, 2). The possible explanation for this failure is a fact that the concentration of both choline and pisatin tested was too low.

In this experiment addition of lipid into medium did not reduce the pisatin sensitivity of *F. solani* f. sp. *pisi* and *P. funerea* (Fig. 1). These data differ somewhat from those of Sweigard and VanEtten[5] who found that addition of lipid into the growth medium reduced the pisatin sensitivity of *Aphanomyces euteiches* and *A. euteiches* f. sp. *phaseoli*. It was suggested that each fungus varied greatly in reactive levels to lipids used. Especially in the relevance to this experiment, Sweigard and VanEtten[5] also failed to show the enhanced pisatin tolerance of *Neurospora crassa* and *F. solani* f. sp. *cucurbitae* when grown on medium supplemented with polar lipids from pea.

The effect of phospholipids on the inhibitive activity of the phytoalexin is interesting phenomenon in the interaction of plant pathogen and the environment which are needed to be further studied to help in improving the strategies of the management on the disease.

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フォスフォリピッドとその関連物質が糸状菌のピサチン感受性に及ぼす影響

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摘　　要

Pisatinの抗菌性に及ぼすphospholipidとそれら構成成分の影響を研究した．Phosphatidylcholine (400 μg/ml) は *Ascochyta pisi, Fusarium solani* f. sp. *pisi, Glomerella cingulata, Cochliobolus miyabeanus* の胞子発芽に対するpisatinの抗菌性を低下させた．また，phosphatidylcholine (400 μg/ml), phosphatidylserine (100 μg/ml) は *Pestalotia funerea, F. solani* f. sp. *pisi* の菌糸伸長に対するpisatinの抗菌性も低下させた．更にphosphatidylcholineの濃度を140 μg/mlから400 μg/mlにすることにより，pisatinの抗菌性は低下することがこれらの菌により認められた．しかし，choline, serine, phosphocholine chloride calcium, total lipidの添加によってはこれらの効果はみられなかった．これらの実験結果から，*in vitro* 及び植物体内において，ファイトアレキシンの抗菌作用にlipidは大きな影響を及ぼすことを示唆している．