

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

Siti Muslimah WIDYASTUTI, Fukuji NONAKA, Nobumichi SAKO
and Eiko MARUYAMA

(Laboratory of Plant Pathology)

Received May 31, 1989

Summary

The effect of phospholipids and their relative components on the pisatin sensitivity of several fungi was studied. Phosphatidylcholine (400 $\mu\text{g/ml}$) decreased the pisatin sensitivity of *Ascochyta pisi*, *Fusarium solani* f. sp. *pisii*, *Glomerella cingulata* and *Cochliobolus miyabeanus* in spore germination test. Decreasing of the pisatin sensitivity of mycelial growth of *Pestalotia funerea* and *F. solani* f. sp. *pisii* was also observed with addition of phosphatidylcholine (400 $\mu\text{g/ml}$) and phosphatidylserine (1,000 $\mu\text{g/ml}$). Increasing of the concentration of phosphatidylcholine from 140 $\mu\text{g/ml}$ to 400 $\mu\text{g/ml}$ 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 lipids¹⁾. Most of lipids are phospholipids²⁾. The four major phospholipids found in all higher cells are phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine and spingomyeline²⁾. 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 processes³⁾. 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. *pisii*, *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 used⁷⁾. Pisatin was added to the germination test water containing 2% ethanol (v/v) and the final concentrations of pisatin were 75 $\mu\text{g/ml}$ and 125 $\mu\text{g/ml}$.

The mycelial growth bioassay. The method used in the bioassay was modification of the method of Pueppke and VanEtten⁸⁾. 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 $\mu\text{g/ml}$. 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. *pisii*, *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

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

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 $\mu\text{g/ml}$) of phosphatidylcholine more reduced the pisatin sensitivity of fungi than that of small one (140 $\mu\text{g/ml}$).

Discussion

Phosphatidylcholine is the major phospholipids in most eukaryotic cell membranes, with phosphatidylethanolamine generally being second most abundant¹⁰. The ability of phospholipids to influence the effectivity of phytoalexins has been previously reported by Bull⁴) with phaseollin and kievitone, and Sweigard and VanEtten⁵) 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 pisatin⁵). In addition it was also possible that phospholipids increased the activity of ATPase¹¹) which is needed for the fungi to overcome the pisatin.

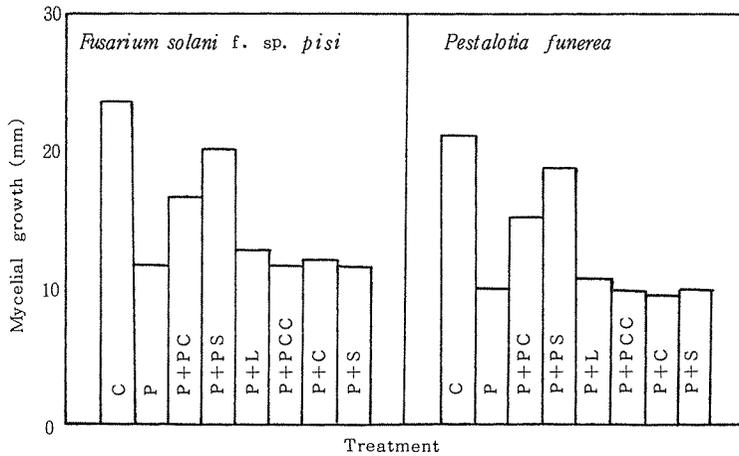


Fig. 1 Effect of phospholipids and their relative components on the pisatin sensitivity of two fungi.

C : Control P : pisatin (75 $\mu\text{g/ml}$)
 P+PC : pisatin+phosphatidylcholine
 P+PS : pisatin+phosphatidylserine
 P+L : pisatin+total lipid
 P+PCC : pisatin+phosphocholine chloride calcium
 P+C : pisatin+choline
 P+S : pisatin+serine
 PC, PS, L, PCC, C and S were used at concentration of 400 $\mu\text{g/ml}$; 980 $\mu\text{g/ml}$; 1,000 $\mu\text{g/ml}$; 990 $\mu\text{g/ml}$; 990 $\mu\text{g/ml}$; and 990 $\mu\text{g/ml}$; respectively

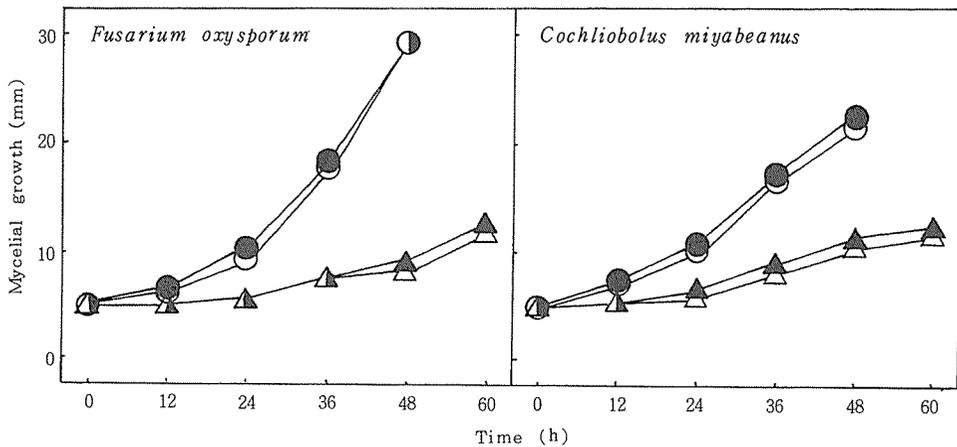


Fig. 2 Mycelial growth of two fungi on sucrose peptone agar without (○) and with serine (●), pisatin (△), pisatin+serine (▲). Serine was added at the final concentration of 1,000 $\mu\text{g/ml}$.

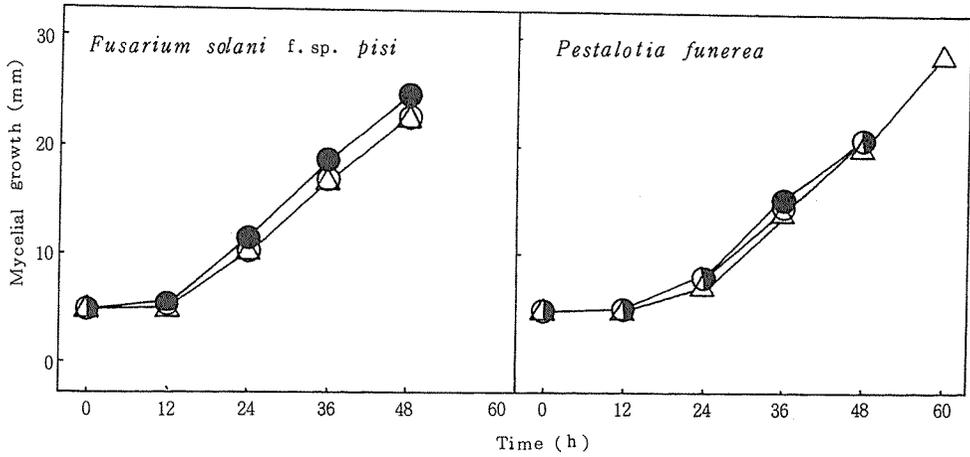


Fig. 3 Mycelial growth of two fungi on sucrose peptone agar without (○) and with phosphatidylcholine (●), choline (△). Phosphatidylcholine were added at the final concentration of 400 $\mu\text{g/ml}$ and 1,000 $\mu\text{g/ml}$, respectively.

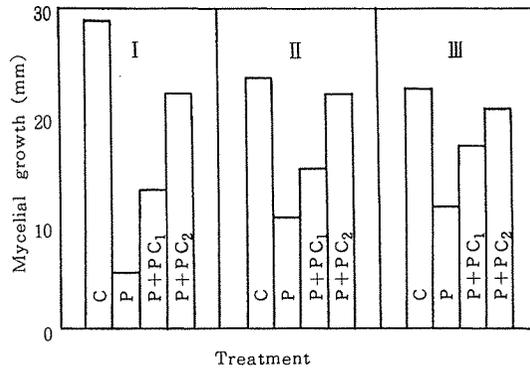


Fig. 4 Effect of different concentrations of phosphatidylcholine on the pisatin sensitivity of three fungi.

I : *Rhizopus stolonifer*

II : *Cochliobolus miyabeanus*

III : *Glomerella cingulata*

C : Control

P : pisatin (75 $\mu\text{g/ml}$)

P+PC₁ : pisatin + phosphatidylcholine (140 $\mu\text{g/ml}$)

P+PC₂ : pisatin + phosphatidylcholine (400 $\mu\text{g/ml}$)

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 VanEtten⁵⁾ 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 VanEtten⁵⁾ 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.

Acknowledgments

Sincerely thanks are due to Dr. T. Yanagita, the Laboratory of Nutrition and Food Hygienic Chemistry for providing lipid, choline and phosphocholine chloride calcium and for his advice during the preparation of this experiments, and to Mr. K. Tanaka, the Laboratory of Plant Pathology for his technical assistant.

Literature cited

1. Ansell, G.B. 1973. Historical introduction. In Form and Function of Phospholipids, Ed. by G.B. Ansell, J.N. Hawthorne and R.M.C. Dawson. Elsevier Scientific Publishing Company, Amsterdam, London, New York.
2. Watson, J.D., Hopkins, N.H., Roberts, J.W., Steiz, J.A. and Weiner, A.M. 1987. Molecular Biology of the Gene Vol. I. The Benjamin/Cummings Publishing Company, Inc.
3. Galliard, T., 1973. Phospholipid metabolism in photosynthetic plants. In Form and Function of Phospholipids, Ed. by G.B. Ansell, J.N. Hawthorne and R.M.C. Dawson. Elsevier Scientific Publishing Company, Amsterdam, London, New York.
4. Bull, C.A. 1981. Studies on the fungitoxicity and relevance to disease resistance of the phytoalexin kievitone. Ph. D. thesis. University of Hull, U.K.
5. Sweigard, J. and H.D. VanEtten. 1987. Reduction in pisatin sensitivity of *Aphanomyces euteiches* by polar lipid extracts. *Phytopathology* 77 : 771-775.
6. Folch, J. 1942. The methods of fractionation of phospholipid. *J. Biol. Chem.* 146 : 36.
7. Kiraly, Z., Z. Klement, F. Solomosi and J. Voros. 1974. *Methods in Plant Pathology*. Elsevier Scientific Publishing Company, Amsterdam, London, New York.
8. Pueppke, S.G. and H.D. VanEtten. 1974. Pisatin accumulation and lesion development in peas infected with *Aphanomyces euteiches*, *Fusarium solani* f. sp. *pisi*, or *Rhizoctonia solani*. *Phytopathology*, 64 : 1433-1440.
9. Tanaka, K. and F. Nonaka. 1983. Effect of certain components of bulb sap on the spore germination

- of *Aspergillus niger*. Trans. Mycol. Soc. Japan. 24 : 205-212.
10. Kinney A.J. and T.S. Moore, Jr., 1986. Phosphatidylcholine synthesis in castor bean endosperm. Plant Physiol. 84 : 78-81.
 11. Kasamo, K. and I. Nouchi. 1987. The role of phospholipids in plasma membrane ATPase activity in *Vigna radiata* L. (Mung bean) roots and hypocotyls. Plant Physiol. 83 : 323-328.

フォスホ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

Siti Muslimah WIDYASTUTI・野中 福次・佐古 宣道・丸山 英子
(植物病理学研究室)
平成元年 5月31日受理

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 $\mu\text{g/ml}$) は *Ascochyta pisi*, *Fusarium solani* f. sp. *pisii*, *Glomerella cingulata*, *Cochliobolus miyabeanus* の孢子発芽に対する pisatin の抗菌性を低下させた。また, phosphatidylcholine (400 $\mu\text{g/ml}$), phosphatidylserine (100 $\mu\text{g/ml}$) は *Pestalotia funerea*, *F. solani* f. sp. *pisii* 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 $\mu\text{g/ml}$ から 400 $\mu\text{g/ml}$ 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。