PHOSPHATIDASES PRODUCED BY SOME FUNGAL RICE PATHOGENS *IN VITRO*[1]

TSUNG-CHE TSENG and LIN-HUAY CHANG[2]

(Accepted July 5, 1970)

Production of extracellular phosphatidases by phytopathogens *in vitro* has been reported in several instances (Mount and Bateman 1969; Oi and Satomura 1963; Pellizzart *et al.* 1970; Tseng and Bateman 1968). This paper reports the abilities of some selected phytopathogens to produce phosphatidases in culture filtrates.

Four pathogenic organisms of rice plant diseases in Taiwan were used in investigation, i. e. *Helminthosporium sigmodium* Cav., *Helminthosporium sigmodium* Cav. var. *irregulare* Cralley et Tullis, *Sclerotium hydrophilum* Saccardo, and *Sclerotium oryzae-sativae* Sawada. All of the pathogens were maintained on potato-dextrose agar at 30 C; and subcultured at one month intervals. Production of phosphatidases was made by growing the tested organisms on rice (*Oryzae sativa* var. Taichung 65) stem medium. To prepare the rice stem medium, healthy stems were harvested from 3 month-old rice plants, either used immediately or stored under −20 C until needed. Twenty five grams of the stem tissues with 10 ml of distilled water in a 500 ml Erlenmeyer flask was autoclaved at 121 C for 30 min. The sterilized medium was then inoculated with a week-old culture of a given organism and incubated at 30 C for two weeks. Cultures were harvested and extracted by grinding in 100 ml of distilled water per cluture in a Virtis '45' homogenizer for 1 min at higher speed and the liquid from the miced materials was strained through four layers of cheesecloth. The extracts were centrifuged at 20,000 *g* for 30 min at 5 C. The supernatants were lyophilized and stored at −20 C in a desiccator until used as enzyme sources.

Phosphatidase activity was detected with the cup plate method as previously described (Tseng and Bateman 1968), except no cofactor was added to the assay

1. The authors are deeply grateful to professors C. C. Chen and L. C. Wu of National Taiwan University, Dr. H. W. Li, Director of the Institute of Botany, Academia Sinica for their encouragements during the course of work.

2. Assistant Research Fellow and Research Assistant of the Institute of Botany, Academia Sinica, Nankang, Taipei, Taiwan, China. Paper No. 101 of the Scientific Journal Series, Institute of Botany, Academia Sinica.

medium. The enzyme activity was expressed as the area of substrate hydolyzed (white zone) per mg nitrogen/0.2 ml of enzyme preparation. Autoclaved enzymes served as controls. Nitrogen contents were estimated by Micro-Kjeldahl Method (Method of analysis. A. O. A. C. 1960).

The results indicated that the culture filtrates of all the organisms contained phosphatidases. Autoclaved enzyme preparations exhibited no detectable phosphatidase activity. When the various enzyme preparations were examined for phosphatidase activity over the pH range 3.5 to 7.5, two buffer systems were used. The pH optima for phosphatidases from the tested organisms were distinctly in the acid range but no enzyme activity has been found for the pH after 7.5 (Fig. 1).

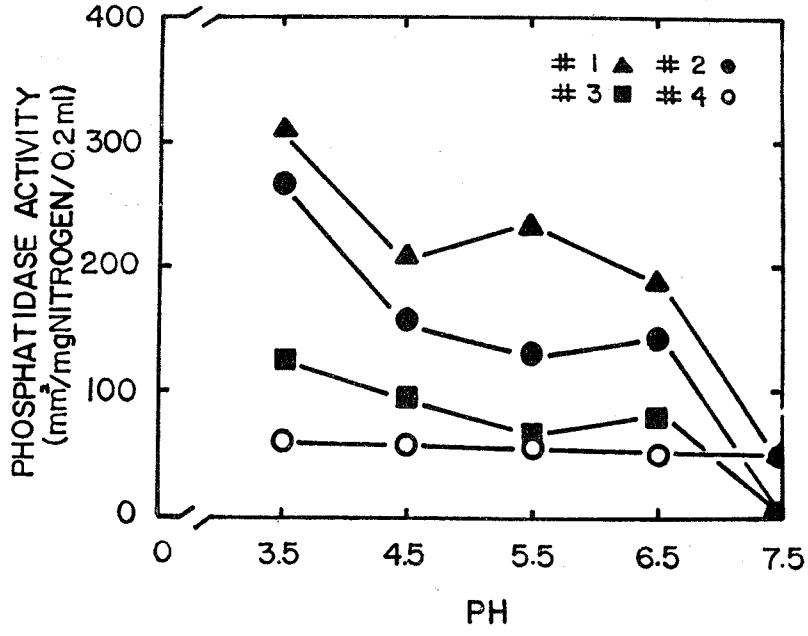

Fig. 1. Effect of pH on phosphatidase activities of some phytopathogens in culture filtrates. Phosphatidase activity was assayed by the cup plate method as described in text. The enzyme activity was expressed as the area of substrate hydrolyzed (white zone) per mg nitrogen/0.2ml of various enzyme sources. Citrate buffer (0.1M) was used for pH 3.5-6.5; phosphate buffer (0.1M) was served for pH 7.0-7.5.
#1=*Helminthosporium sigmodium* Cav.
#2=*Sclerotium oryzae sativae* Sawada
#3=*Helminthospium sigmodium* Cav. var. *irregulare* Cralley et Tullis
#4=*Sclerotium hydrophilum* Saccardo

Literatures relating phosphatidases to plant diseases are scanty. The occurrances of phosphatiadses in *Sclerotium rolfsii*-infected bean hypocotyls (Tseng and Bateman 1968) and *Thielaviopsis basicola*-infected bean roots (Lumsden

and Bateman 1968) appear to be the only reports indicating that phosphatidases of pathogens origin are associated with plant pathogenesis. However, in those instances the role of phosphatidases per se has not been elucidated. Our preliminary studies with *Corticium sasakii* (Shir) Mats. infected with rice shealth have demonstrated the existence of phosphatidases in disease tissues (unpublished data). Further investigations of these organisms with respect to the occurance and the catalytic actions of phosphatidases in disease tissues that may provide a better understanding for the host-parasite relationships.

Literature cited

LUMSDEN, R. D., and D. F. BATEMAN. 1968. Phosphatide degrading enzymes associated with pathogenesis in *Phaseolus vulgaris*-infected with *Thielaviopsis basicola*. Phytopathology **58**: 219-227.

Method of analysis. A. O. A. C. 1960. Microchemical method—nitrogen. p. 643-644.

MOUNT, M. S., and D. F. BATEMAN. 1969. Production of phosphatidase C by *Erwinia carotovora*. Phytopathology **59**: 401.

OI, S., and Y. SATOMURA. 1963. Purification and substrate specificities of phosphatidase A and B of *Sclerotium* fungus. Agr. Biol. Chem. **27**: 397-404.

PELLIZZARI, E. D., J. KÚE, and E. B. WILLIAMS. 1970. Hydrolytic activity in supernatant from germinated spores of *Venturia inaequalis*. Phytopathology **60**: 389-390.

TSENG, T. C., and D. F. BATEMAN. 1968. Production of Phosphatidases by phytopathogens. Phytopathology **58**: 1437-1438.

TSENG, T. C., and D. F. BATEMAN. 1969. A phosphatidase produced by *Sclerotium rolfsii*. Phytopathology **59**: 359-363.