

# ジャガイモ細胞のリシチン合成,代謝経路の活性に対するジャガイモ疫病菌細胞壁成分の影響

誌名	日本植物病理學會報 = Annals of the Phytopathological Society of Japan
ISSN	00319473
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巻/号	47巻2号
掲載ページ	p. 255-257
発行年月	1981年4月

## Effect of Hyphal Wall Component of *Phytophthora infestans* on Activity of Rishitin Synthetic Pathway in a Potato Cell Exposed Directly to the Component

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酒井 進\*・富山宏平\*・道家紀志\*：ジャガイモ細胞のリシチン合成、  
代謝経路の活性に対するジャガイモ疫病菌細胞壁成分の影響

A previous paper<sup>1)</sup> reported that the rishitin synthetic pathway was activated in the healthy tissue about 2 mm in thickness adjacent to the cut surface tissue of potato tuber infected by an incompatible race of *P. infestans* within 2 days after cutting and inoculation.

In another study<sup>2)</sup>, we reported that the rishitin synthetic pathway had been under operation in tissue slices of potato tuber buds and also in those of shoots sprouted from potato tubers within 10 min after slicing. These results suggested that the rishitin synthetic pathway may operate in amount detectable with an isotope in an intact young potato tissue which is metabolically active.

These findings imply that the onset of rishitin synthesis does not need stress such as infection, or treatment with chemicals or hyphal wall components (HWC) and so on. Many reports<sup>3,4)</sup> have indicated that hyphal wall components (HWC) of pathogenic fungi elicited accumulation (or production) of phytoalexins.

These rather contradictory findings pose a question as to whether HWC accelerate the activity of an active rishitin synthetic pathway or not.

In the experiments reported here, we tried to clarify whether HWC activates or inhibits the synthetic pathway of rishitin in a cell exposed directly to HWC.

Tubers of the potato cultivar Rishiri were stored at 4 C and kept at 18 C for 48 hr before use. Tissue cylinders, 16 mm in diameter, were removed from the central parenchymatous tissue with a cork borer. Disks 0.5 mm thick were sliced from the cylinders with a microtome and washed with a large amount of distilled water. The disks were incubated in a plastic box at 18 C for 3 hr and then the disks were immersed in 1 ml of distilled water containing 5  $\mu$ Ci of acetate-2-<sup>14</sup>C (sp. activity 58 mCi/mole) for 1 hr. After treatment, the disks were washed with distilled water three times and then excess water was blotted off. Immediately after the treatment with acetate-2-<sup>14</sup>C, the washed disks were immersed in HWC solution (1 mg/ml H<sub>2</sub>O) or distilled water as a control for 5 min. The HWC was isolated from mycelium of *P. infestans* (race 3) cultured in rye seed extracts medium<sup>5)</sup> according to the modified method<sup>6)</sup> of Lisker and Kuć<sup>4)</sup>. After the

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1) Nakajima, T., Tomiyama, K. and Kinukawa, M. (1975). Ann. Phytopath. Soc. Japan 41: 49-55. 2) Sakai, S., Tomiyama, K. and Doke, N. (1979). Ann. Phytopath. Soc. Japan 45: 705-711. 3) Albersheim, P. and Valent, B. S. (1978). J. Cell Biol. 78: 627-643. 4) Lisker, N. and Kuć, J. (1977). Phytopathology 67: 1356-1359. 5) Caten, C. E. and Jinks, J. L. (1968). Can. J. Bot. 46: 329-348. 6) Doke, N. and Tomiyama, K. (1980). Physiol. Plant Pathol. 16: 169-179.

treatment, the tuber disks (1.1 g) were used for determination of rishitin contents.

Potato tissues were immersed in 25 ml of MeOH, 0, 1, 3 and 5 hr after the treatment with HWC and then kept for 24 hr. The MeOH was sampled and then the tissues were again immersed in renewed MeOH for 24 hr. The MeOH-extracts were combined (50 ml), condensed to syrup under vacuum, and the syrup was extracted three times with 2 ml of EtOAc. An appropriate amount of non-radioactive rishitin was added to EtOAc extract as a carrier and then the rishitin fraction was separated by repeating four different TLC according to the method reported previously<sup>2)</sup>. The solvents used for the developments were cyclohexane: ethyl acetate (1:1, v/v) [1st TLC], ether [2nd TLC], EtOAc: MeOH (80:20, v/v) [3rd TLC] and EtOAc [4th TLC]. Radioactivity of the rishitin peak was determined using 10 ml scintillation fluid according to the method of Ishiguri *et al.*<sup>7)</sup>

When the fresh tuber disks (3 hr after preparation), which had been treated with acetate-2-<sup>14</sup>C, were treated with HWC, the increase in incorporation of radioactivity into rishitin with time was inhibited by HWC treatment. Experiments were repeated three times and always similar results were obtained (Fig. 1). In Fig. 1, averages of the experiments are shown, and the arrows indicate maximum and minimum values.

The thin disks 0.5 mm thick used in the present experiments consisted of 2.3 cells layer in average. Therefore, almost all of the cells were exposed directly to HWC. Microscopic observation showed that no cell death occurred by 12 hr after the treatment with HWC under the present experimental condition.<sup>8)</sup>

The present results showed that HWC reduced accumulation of <sup>14</sup>C-labeled rishitin in a cell which was exposed directly to HWC, although it was not certain whether HWC inhibited the synthesis and metabolism (transformation) of rishitin<sup>7,9)</sup> at the same rate or differently.

GLC-analysis of rishitin<sup>8)</sup> showed that distinct accumulation of rishitin began after cell death caused by HWC appeared about 12 hr after the treatment with HWC. The previously reported results<sup>7,8,9,10)</sup> suggest that the rishitin synthetic pathway operates in

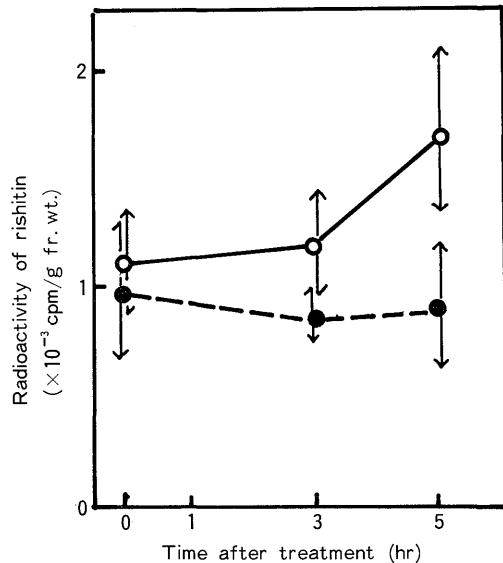


Fig. 1. Effect of hyalal wall component (HWC) of *P. infestans* on the incorporation of radioactivity from acetate-2-<sup>14</sup>C into rishitin in the fresh potato tuber disks. HWC treatment (●), water treatment (○). Arrows indicate maximum and minimum values.

7) Ishiguri, Y., Tomiyama, K., Doke, N., Murai, A., Katsui, N., Yagihashi, F. and Masamune, T. (1978). *Phytopathology* 68: 720-725. 8) Sakai, S., Tomiyama, K. and Doke, N. (1979). *Ann. Phytopath. Soc. Japan* 45: 107 (Abstr.). 9) Horikawa, T., Tomiyama, K. and Doke, N. (1976). *Phytopathology* 66: 1186-1191. 10) Ishizaka, N. and Tomiyama, K. (1972). *Plant & Cell Physiol.* 13: 1053-1063.

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metabolically active tissue such as intact young tissue or healthy tissue adjacent to cut or infected ones of potato plants. HWC may reduce activity of rishitin accumulation in a living cell which is exposed directly to HWC, but accumulation of rishitin occurs in the dead cells after HWC induced dead tissue zone in the potato tissue<sup>8)</sup>.

(Received November 10, 1980)