

**Effect of yam chitinase against the powdery mildew,
Sphaerotheca humuli, infecting on strawberry**

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Abstract

To investigate whether chitinase, a plant self-defense enzyme, is effective against the powdery mildew, *Sphaerotheca humuli* de (de Candolle) Burrill, infecting on plants, yam chitinase E (family 19, class IV) was directly sprayed onto the infected berries and leaves of strawberry, *Fragaria* × *ananassa* Duchese, Toyonoka, at a concentration of 0.5 - 5 mg/50 ml. The effect of the chitinase on the pathogens was followed visually and with a scanning electron microscope within a time period of one week. The results showed that the hyphae of the strawberry powdery mildew were degraded due to the action of the chitinase, suggesting that yam chitinase E (family 19, class IV) is potent against plant pathogens and can be applied as a biocontrol agent.

Introduction

Toyonoka is the most popular strawberry cultivar in Japan, and susceptible to many fungal diseases, especially the powdery mildew, *Sphaerotheca humuli* (de Candolle) Burrill. We purified several chitinase isozymes from the tuber of yam, *Dioscorea opposita* THUNB¹⁾. Among them, yam chitinase E, which is classified into the family 19 of glycosyl hydrolases and class IV, has strong lytic activity against *Fusarium oxysporum*¹⁾. In this study, we investigated the effect of yam chitinase E against the powdery mildew, *S. humuli*, infecting on plants in a greenhouse by directly spraying onto the surface of the infected leaves and berries of strawberry, *Fragaria* × *ananassa* Duchese, Toyonoka. The lethal effect of the chitinase against the powdery mildew was observed visually and using a scanning electron microscope.

Methods

Enzymes Chitinase E was purified from the tuber of yam, *Dioscorea opposita* THUNB, by the method of Arakane *et al*¹⁾. Zymolyase 20T, which contains mainly β -1,3-glucanase, was obtained from commercial source (Seikagaku Corporation, Japan).

Powdery mildew inoculation and enzymes treatment The strawberry, *Fragaria* \times *ananassa* Duchese, Toyonoka was raised in a greenhouse. Three months after germination the berries and leaves were inoculated with powdery mildew spores suspended in a dilute nonionic detergent (polyoxyethylene nonylphenyl ether) solution. Chitinase E (0.5 mg/50 ml and 5 mg/50 ml), Zymolyase-20T (0.3 g/50 ml), or enzyme mixture (5 mg/50 ml chitinase E and 0.3 g/50 ml Zymolyase 20T), which were dissolved in the dilute nonionic detergent solution, were directly sprayed to the infected berries and leaves.

Observation with scanning electron micrograph (SEM) Preparation of samples for SEM was done by the method of Tanaka *et al*²⁾.

Results

The white powder on the surfaces of the treated berries and leaves disappeared one day after sprayed with any solutions. When treated with chitinase E (5 mg/50 ml) only or the enzyme mixture, the degraded hyphae were observed with a scanning electron microscope (SEM). Even one week later, the powdery mildew did not recover to grow on the berries and leaves, whereas the treatment with Zymolyase 20T only could not stop completely the growing of powdery mildew. However, Zymolyase 20T seemed to act synergistically with chitinase against powdery mildew. On the other hand, in the control experiment the hyphae of the pathogen were growing with intact surface.

Reference

- 1, Arakane *et al.*, *Biosci. Biotechnol. Biochem.* **64**, 723-730 (2000).
- 2, Tanaka *et al.*, *Can. J. Bot.* **57**, 2528-2532 (1979).

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