The Testing of TiO_2 Photocatalityc Reaction to Inhibit of *Botryotinia fuckeliana*

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Abstract

We studied the antifungal activity of the titanium dioxide (TiO_2) photocatalytic reaction, which shows antibacterial effects under UVA (315 - 400 nm) irradiation, against *Botryotinia fuckeliana*. Under the UVA irradiation of 1.0 mW/cm, growth of *B. fuckeliana* treated with TiO_2 solution was inhibited 55% more than that treated with sterile distilled water (SDW). Its effectiveness was almost same as chlorothalonil, a commercial fungicide. Furthermore, even TiO_2 solution at lower concentration showed antifungal activity by TiO_2 photocatalytic reaction under UVA irradiation, since growth of *B. fuckeliana* treated with TiO_2 solution at 8.5×10^{-2} mg/l was significantly decreased compared to that treated with SDW. These results suggested that the TiO_2 photocatalytic reaction might be available as a novel fungicide against phytopathogens generated before harvesting such as *B. fuckeliana*.

Keywords: Titanium dioxide photocatalytic reaction, *Botryotinia fuckeliana*, Chlorothalonil, UVA irradiation

1. Introduction

In recent years, consumers have shown a growing interest in food safety. To date, agricultural crops have been supplied stably and effectively by using chemical pesticides, but there may be a risk of mammalian toxicity and environmental pollution by the chemical pesticides. Such chemical products are a health hazard to farmers and consumers, and remain in the crops. The alternatives such as organic farming or pesticide-free production may be time-consuming and incur damage from the phytopathogens and pests.

Titanium dioxide (TiO_2) has a photocatalytic reaction under UVA (315 - 400 nm) irradiation and shows many excellent effects such as disinfection (Maness *et al*., 1999; Ibáñez *et al*., 2003; Kim *et al*., 2003; Maneerat and Hayata, 2006; Chawengkijwanich & Hayata, 2008) and decomposition (Maneerat *et al*., 2003), and can be used for a deodorant (Murakami *et al*., 2004) and water purification (Herreara Melian *et al*., 2000; Wist *et al*., 2002), but there are few reports on agricultural crops (Lu *et al*., 2006). When activated by UVA irradiation, the TiO_2 photocatalytic reaction generates hydroxyl radicals and superoxide ions at the TiO_2 surface. The hydroxyl radicals are used as a powerful oxidizing agent to convert organic pollutants absorbed on the TiO_2 surface into CO_2 and water vapor (Fujishima and Honda, 1972; Fujishima *et al*., 2000). In present study, we therefore compared the antifungal activity of the TiO_2 photocatalytic reaction on *Botryotinia fuckeliana* with common chemical pesticides. *B. fuckeliana* is one of the airborne phytopathogens with a necrotrophic lifestyle attacking over 200 crop hosts worldwide (Williamson *et al*., 2007).

2. Materials and Methods

2.1 Preparation of TiO_2 and Chlorothalonil Solutions

TiO_2 solution (8.5×10^2 mg/l Miracle titania M solution; a mixture of peroxotitanium acid solution and peroxo-modified anatase solution, Ohno Sekiyuten Co. Ltd., Hiroshima, Japan) was diluted with sterile distilled water (SDW) and used for the experiments.

Chlorothalonil (active ingredient: 40% tetrachloroisophthalonitrile, Sumitomo Chemical Garden Products Inc., Tokyo, Japan), a commercial fungicide, was diluted with SDW and used for the experiments as a positive control.

2.2 Measurements of Antifungal Activity

Botryotinia fuckeliana (NBRC9760) was purchased from the National Institute of Technology and Evaluation (Kisarazu, Japan). One ml of TiO_2 solution, SDW or chlorothalonil was spread on potato dextrose agar (PDA) plates, the surfaces of which were aseptically air-dried. A fraction of agar containing *B. fuckeliana* (0.4 cm in diameter) was plated at the center of the PDA plates (8.5 cm in diameter) and cultured at 25°C under UVA (315 - 400 nm) irradiation from 20-W black light lamps for 7 days. The UVA irradiation intensity from the 20-W black light lamps was measured at 0.0 (no irradiation), 0.3, 0.5 and 1.0 mW/cm during the experiments by a UVR-400 radiometer (S-365 UV-sensor, wavelength sensitivity of 320 - 410 nm, Iuchi, Osaka, Japan). After irradiation, the colony diameter of grew *B. fuckeliana* was measured with vernier calipers (Fig. 1).

2.3 Statistical Analysis

All experiments were performed in triplicate. The data presented are the means with standard errors. The experimental data were evaluated by analysis of variance (ANOVA), and the significant difference was analyzed using the Turkey-Kramer method at $p<0.05$.

3. Results

Inhibitory effect of chlorothalonil on the colony growth of *B. fuckeliana* is shown in Figure 2. Antifungal activity increased with the concentration of chlorothalonil, and there was significantly antifungal activity at the concentration of chlorothalonil more than $1.0 \times 10^{2.6}$ mg/l. The level is commonly-used concentration. Therefore, antifungal activity of TiO_2 photocatalytic reaction against *B. fuckeliana* was compared with that of $1.0 \times 10^{2.6}$ mg/l chlorothalonil.

Inhibitory effect of UVA irradiation on the colony growth of *B. fuckeliana* by TiO_2 photocatalytic reaction is shown in Figure 3. With the SDW treatment, the growth of *B. fuckeliana* was inhibited 18% at 1.0 mW/cm^2 of UVA irradiation, although there were no significant differences in the growth at the range of UVA irradiation intensity less than 0.5 mW/cm^2. In contrast, the growth of *B. fuckeliana* treated with TiO_2 was inhibited significantly under all levels of UVA irradiation. Growth of *B. fuckeliana* treated with TiO_2 solution under 0.3, 0.5, and 1.0 mW/cm^2 of UVA irradiation decreased approximately 34, 40, and 55%, respectively, compared to non-treated *B. fuckeliana*, which grew similarly under all levels of UVA irradiation intensity. The TiO_2 photocatalytic reaction showed a good ability to inhibit the growth of *B. fuckeliana* comparable to that of chlorothalonil at $1.0 \times 10^{2.6}$ mg/l, an adequate concentration, when used TiO_2 solution under 1.0 mW/cm^2 of UVA irradiation.

Inhibitory effect of TiO_2 at various concentrations on the colony growth of *B. fuckeliana* by TiO_2 photocatalytic reaction is shown in Figure 4. The antifungal activity of the TiO_2 photocatalytic reaction against *B. fuckeliana* increased concomitant with the concentration of TiO_2 solution. Growth of *B. fuckeliana* was inhibited approximately 55, 54, 48, 40, and 34% by photocatalytic reaction with TiO_2 solution diluted to 8.5×10^2 (no dilution), 8.5×10^1, 8.5×10^0, 8.5×10^{-1}, and 8.5×10^{-2} mg/l, respectively, compared to non-treated *B. fuckeliana*, which grew similarly under all levels of UVA irradiation intensity. These responses clarified that the TiO_2 photocatalytic reaction was highly efficient for inhibiting the growth of *B. fuckeliana*.

4. Discussion

The TiO_2 photocatalytic reaction has an antibacterial activity on many bacteria causing food poisoning (Kim *et al*., 2003; Kuhn *et al*., 2003). The antifungal activity of TiO_2 photocatalytic reaction against phytopathogens that might affect postharvest tomatoes and kiwifruit has also been tested (Hur *et al*., 2005; Maneerat and Hayata, 2006). Furthermore, inactivation of *Fusarium oxysporum* and *Pythium aphanidermatum* in hydroponic solution by TiO_2 photocatalytic reaction have been studied (Nakabayashi *et al*., 1999; Koohakan *et al*., 2003). In present study, the antifungal activity of TiO_2 photocatalytic reaction against *B. fuckeliana* was highly effective at UVA irradiation intensity more than 1.0 mW/cm^2. The antifungal activity may be of great use in the agricultural field, since UVA irradiation intensity contained in the sunlight near the equator is approximately 2.0 mW/cm^2 (Joyce *et al*., 1996; Lonnen *et al*., 2005). The results of present study are suggested to have raised the possibility of using the TiO_2 photocatalytic reaction before harvesting at agricultural field. Moreover, phytopathogens may get tolerant on chemical bactericides, but appear to gain no resistance to the TiO_2 photocatalytic reaction, since the antibacterial activity of the TiO_2 photocatalytic reaction occurs with oxidation. TiO_2 is nontoxic and has been approved by the American Food and Drug Administration for use in human food, drugs, and cosmetics, and

compounded in food contact materials such as cutting boards and other surfaces that come in contact with unprotected food. Furthermore, it is safe and nontoxic with excellent durability, and is used in toothpaste and cosmetics, and is approved as food additive (Taoda, 2009).

5. Conclusion

The TiO_2 photocatalytic reaction at UVA irradiation more than 1.0 mW/cm^2 exhibited a high antifungal activity comparable to that of chlorothalonil, a commercial fungicide. The effectiveness increased with the concentration of TiO_2 solution. It is suggested that TiO_2 is available as a novel fungicide against phytopathogens generated before harvesting such as *B. fuckeliana*. In future, to introduce TiO_2 photocatalytic reaction into actual agricultural field, it is necessary to do in vivo experiment.

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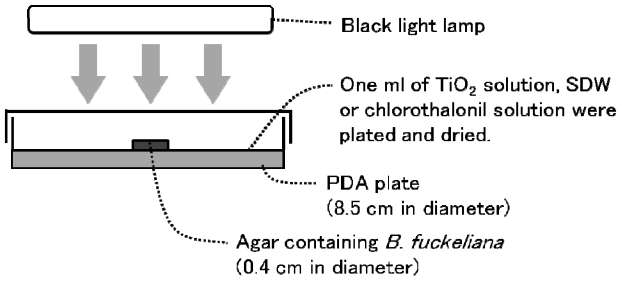

Figure 1. The evaluation method of the antifungal activity

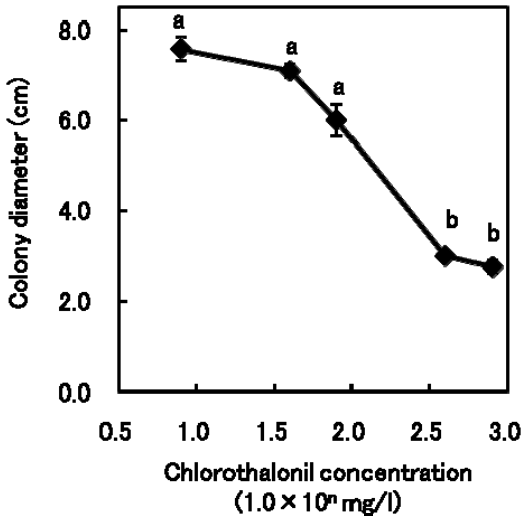

Figure 2. Antifungal activity of chlorothalonil at various concentrations on the colony growth of *B. fuckeliana*. Vertical bars indicate the standard error. Different letters indicate a significant difference (p‹0.05)

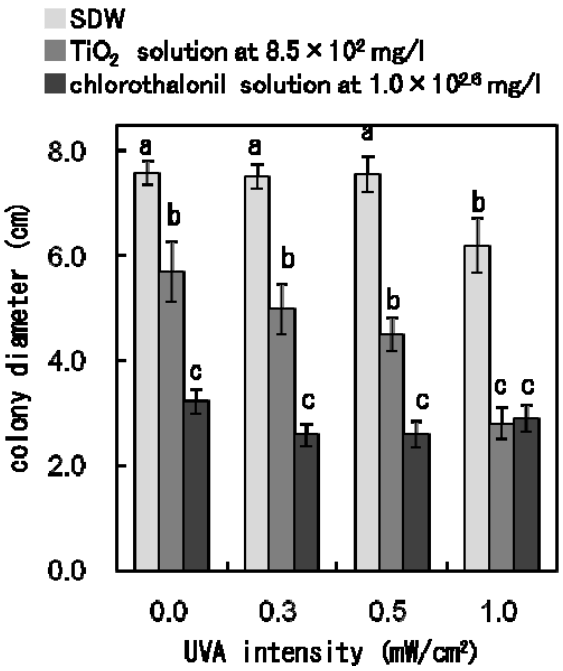

Figure 3. Effect of UV irradiation on inhibiting the colony growth of *B. fuckeliana* by TiO_2 photocatalytic reaction

Vertical bars at the top of the shaded bars indicate the standard error. Different letters indicate a significant difference (p‹0.05).

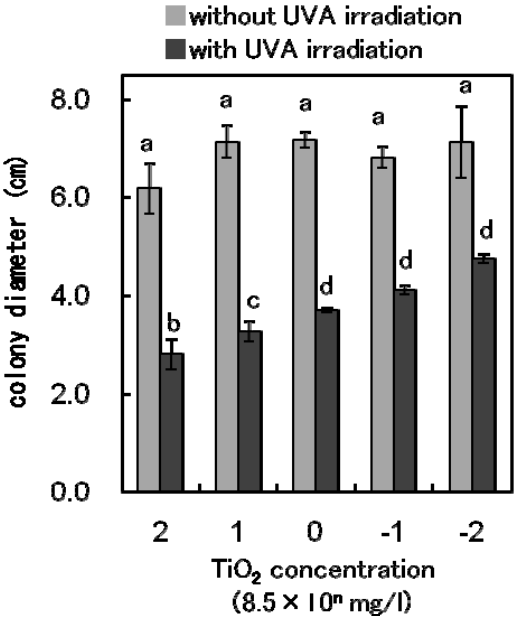

Figure 4. Effect of TiO_2 solution at various concentrations on inhibiting the colony growth of *B. fuckeliana* by TiO_2 photocatalytic reaction

Vertical bars at the top of the shaded bars indicate the standard error. Different letters indicate a significant difference (p‹0.05).

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