Effect of Near-Ultraviolet Irradiation on Sporulation by *Pyricularia oryzae* Cavara on Cultural Media

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におよぼす近紫外線照射の影響

Abstract

Effects of near-ultraviolet irradiation on the sporulation of *Pyricularia oryzae* Cavara were investigated by daily count of conidial formation of the fungus grown at $27 \pm 1^\circ\text{C}$ on potato sucrose agar inoculated uniformly flooded with fungal suspension. Continuous irradiation with near-UV (366 nm) on 4 isolates of *P. oryzae* generally shortened the time required to reach maximum sporulation, but the effect of continuous irradiation on the amounts of sporulation varied among isolates. Irradiation for the first 3 days resulted the highest sporulation and accelerated conidiophore formation. Sporulation, however, was less in continuous darkness or in darkness for the first 3 days. Near-UV absorbing, water-soluble and heat-unstable substances with maximum absorption at 325 nm were detected from the cultures of an isolate irradiated for 4 days, but not from unexposed cultures. When the irradiated fungal extracts were added to medium, sporulation under irradiation increased remarkably. In darkness, both fresh and autoclaved fungal extracts depressed sporulation.

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Introduction

The knowledge about the process of sporulation will lead us to find not only the effective way of control in the sense of epidemics but also the effective way of obtaining enough inoculum timely for various purposes in laboratory.

Studies on sporulation of *Pyricularia oryzae* Cavara, including the effect of light and artificial media, have been reviewed by Furuta *et al.*³⁾ and Suzuki²⁰⁾. But works on the effect of near-ultraviolet irradiation on sporulation of *P. oryzae* have been quite limited^{10,16)}. Near-ultraviolet has been found to affect sporulation of many fungi^{2,9,10)}. According to Ohmori *et al.*¹⁶⁾, 340 nm and 365 nm wave lengths were found to be effective in sporulation of *P. oryzae*. On the other hand, length of irradiation and timing in relation to growth stage have been found to be associated with the effect of irradiation on sporulation of *P. oryzae*^{1,3,15)}. During the process of sporulation, irradiation, especially in the near-UV portion of the spectrum, has been known to induce conidiophores but to suppress conidial development in *P. oryzae*⁷⁾, *Helminthosporium teres*¹⁷⁾, *H. oryzae*⁵⁾ and *Alternaria dauci*²³⁾.

Although there have been suggested many possible modes of action of light²²⁾, many things still remain to be investigated. Some investigators have tried to detect some photoreceptors which

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may be involved in light-induced sporulation^{11,12)}, and reported the isolation of sporogenic substances from fungi²¹⁾. In *P. oryzae*, no study has been done about the presence of certain substances within fungal colony in relation to light-induced sporulation.

Therefore, the present study was done to investigate the effect of near-UV irradiation on sporulation and to ascertain the presence of substances absorbing near-UV which might play an important role in photo-induced sporogenesis in *P. oryzae*.

Materials and Methods

All of the isolates of *Pyricularia oryzae* Cavara were grown on potato sucrose agar (PSA) added with streptomycin sulfate in order to avoid bacterial contamination and kept them at $27 \pm 1^\circ\text{C}$ in an incubator, unless otherwise stated.

As light source, the long wave length ultraviolet lamp, Mineralight M/UVSL-25 (wave length mainly 366 nm), was used for most of this study. For fungal extraction, a Matsushita 20-W black light blue fluorescent lamp (FL 20S.BL-B, wave length 300-450 nm) was used.

Cultures were irradiated through Pyrex glass petri dishes with a distance of 18 cm from lamp to culture, unless otherwise indicated. The number of conidia was counted in a hemacytometer. All the experiments were made with three replicates except fungal extraction. Analysis of variance was used to help to interpret the data. Differences between means were evaluated using Duncan's new multiple range test.

Isolates: The isolates of *P. oryzae* were obtained from the Institute of Plant Environment, Office of Rural Development. Four isolates showing distinctive reactions under continuous irradiation were selected through the preliminary test. The isolates used (race designation in parenthesis) were Oh-A16 (T-1), 9h-153 (N-1), H-774 (N-3), Oh-A4 (C-8).

Inoculation: The inoculum was produced on PSA after 10 days' incubation. Each petri dish, containing 20 ml of PSA, was inoculated uniformly by flooding the agar surface with 0.5 ml of spore and mycelial suspension made by crushing the culture of three 2 cm² discs from one petri dish in 8 ml of distilled water for 90 seconds using an electric shaver (National, 1.5 volt D.C.) attached with edged rotatory wire.

Spore counting: Spores were counted at 24-hr intervals from the start of exposure unless otherwise indicated. Samples were taken in each time from random locations in each dish. The number of spores formed in each time was recorded separately by not adding the sum of spores being formed in that age of the culture. One disc was cut by a biscuit cutter (5 mm in diameter), then crushed in a vial containing 4 ml of water. After adding 2 drops of 10% formalin for fixation in the vial, 6 samples from each vial were counted.

Fungal extraction and spectroscopic examination: Isolate H-774, produced significantly more conidia in light than in darkness, was used in this study. Fungal extraction was made by a modification of the method described by Leach¹²⁾. Eight petri dishes were seeded with the fungal suspension onto cellophane (9 cm in diameter, 0.05 mm in thickness) overlaying PSA. An additional 8 dishes were similarly prepared and kept in darkness. Cultures were exposed to light for 4 days continuously. Eight g of wet culture, both from light-treated and from control cultures was peeled from the cellophane, crushed in 100 ml of cold (5°C) distilled water with a blender, and kept overnight in a refrigerator at 5°C. Then the extracts were filtered through No. 5B Toyo filter papers. Each filtrate was centrifuged at 12,000 rpm for 30 minutes and the supernatant was taken. This supernatant was shaken with the same volume of petroleum ether, and both phases were examined in the Beckman DU-2 spectrophotometer with 10 nm of band width from 200 nm to 900 nm. Near

the maximum absorption peak a 5 nm band width was used.

Bioassay of irradiated fungal extracts: To examine the effect of irradiated fungal extracts on sporulation, the extracts were filtered through a Seiz filter and half the volume of each was autoclaved at 121°C (15 lbs pressure) for 20 minutes. Bioassay was performed with both autoclaved and nonautoclaved extract. In each case, half the volume of water in PSA was replaced with the fungal extract before pouring into petri dishes. For a control, distilled water was added to the medium instead of fungal extract. The media thus treated were flooded with fungal suspension. Half of the cultures thus prepared were then irradiated, the other half kept in darkness for 5 days.

Results

Effect of continuous irradiation: Conidial formation by the four isolates growing on PSA under continuous light and continuous darkness was observed daily (Fig. 1).

Fig. 1. Effect of continuous irradiation with near-UV on daily sporulation of four isolates of *Pyricularia oryzae* on potato sucrose agar.

Conidia were produced in both light and darkness. Generally, light appeared to hasten the sporulation process of each isolate. But the effect of continuous near-UV irradiation on the amount of conidia produced was different. Especially, isolate H-774 showed remarkably accelerated sporulation and the largest number of conidia in light was greater than that in darkness, the differences being statistically significant ($P=0.99$). In contrast, isolate Oh-A 16 produced more conidia in darkness than in light, and the differences were also statistically significant ($P=0.99$). However, the numbers of conidia produced by isolates 9h-153 and Oh-A4 under the two treatments were not significantly different, although light also appeared to accelerate the sporulation. In all of the isolates, sporulation rose to a maximum and then decreased. Therefore, in the succeeding experiments, comparison of sporulation was made when the maximum sporulation occurred in the same light condition.

Effect of irradiation period: Test of the time at which cultures are most sensitive to light were made with 2 isolates, Oh-A 16 and H-774. Four treatments were used: continuous irradiation, continuous darkness, irradiation for the first 3 days after seeding followed by darkness, and darkness for the first 3 days followed by irradiation. Comparisons were based on conidial counts at the time of maximum sporulation under continuous irradiation: 7 days for Oh-A 16, 5 days for H-774 (Fig. 1).

Table 1. Sporulation by *Pyricularia oryzae* on potato sucrose agar exposed to near-UV on different days

Light treatment	10 ³ conidia/ml ^{a)}	
	Oh-A16	H-774
Continuous darkness	24.7 b	7.3 a
Irradiation for first 3 days	50.3 c	122.7 d
Darkness for first 3 days	1.7 a	22.7 b
Continuous irradiation	1.0 a	109.3 c

a) Average of three replicates in each of two experiments. Conidia were counted after 7 days for Oh-A16, 5 days for H-774. Numbers followed by different letters are significantly different from each other at 1%.

Both isolates produced the greatest number of conidia when the cultures were irradiated only for the first 3 days after seeding (Table 1). The cultures exposed to light 3 days after seeding produced much fewer conidia. Sporulation under continuous irradiation was somewhat depressed as compared to those under irradiation for first 3 days, especially more strikingly in the isolate Oh-A16.

Absorption spectra of fungal extracts: The presence of near-UV absorbing substances in the fungal colony was investigated with the isolate H-774. Three days after seeding, parts of colony cut from the culture were examined under the microscope.

It was found that conidiophores, some with conidia, were formed in the culture grown in light, but not in that grown in the dark (Fig. 2). The absorption spectra of the petroleum ether-soluble and water-soluble components of the extracts from irradiated and unexposed cultures were compared. The absorption spectra of the petroleum ether-soluble components of the irradiated cultures did not differ from those of the unexposed cultures. But the water-soluble components from each

Fig. 2. *Pyricularia oryzae* grown on potato sucrose agar for 3 days under near-UV irradiation (L) and darkness (D). $\times 170$

treatment showed different absorption spectra in the region of wave length between 300 and 360 nm (Fig. 3). The extracts from the irradiated cultures showed strong absorption peak with maximum at 325 nm.

Effect of fungal extracts on sporulation: To examine the role of fungal extracts in sporulation, isolate H-774 was seeded on PSA to which had been added with; (1) autoclaved fungal extracts; (2) non-autoclaved fungal extracts; (3) as a control, distilled water. Cultures grown on each medium were incubated (1) under light and (2) in darkness.

Under light, the culture grown with nonautoclaved fungal extracts showed significantly higher sporulation than the control, but this was not true of the culture grown with the autoclaved fungal extracts (Table 2). In the culture grown in the dark, those with both the autoclaved and the nonautoclaved fungal extracts showed less sporulation than the control. The interaction between light and fungal extracts was statistically significant ($P=0.99$).

Fig. 3. Absorption spectra of water phase of fungal extracts of 4 day-old colonies of *Pyricularia oryzae* (isolate H-774) grown under light and in darkness.

Table 2. Sporulation by isolate H-774 of *Pyricularia oryzae* on potato sucrose agar containing water soluble fungal extracts from irradiated colonies

Light treatment	10 ³ conidia/ml ^{a)}		
	Nonautoclaved	Autoclaved	Control
Light	197.0 b	130.7 a	115.0 a
Darkness	13.2 a	16.8 a	27.3 b

a) average of three replicates in each treatment.

Differences due to light, extract, and interaction of light×extract were statistically significant at 1%.

Discussion

It has been found that *P. oryzae* produces more conidia under near-UV irradiation than in darkness^{10,16)}, though nothing has been known as to how near-UV radiation affects the time pattern of sporulation.

As noted in another study⁴⁾, light also accelerated the sporulation in this experiment. Also in accordance with other studies^{1,16)}, the effect of light on the amount of sporulation differed among isolates. In most previous studies, sporulation has been simply examined after a certain period of incubation without considering the time pattern of each isolate under given conditions, thus the important characteristic of dynamic role of light, especially of near-UV radiation, has been overlooked.

Irradiation for the first 3 days was most effective in sporulation. Subsequent irradiation,

however, caused virtual cessation of sporulation both in cultures initially irradiated or unirradiated. On the other hand, the formation of conidiophore was stimulated by near-UV irradiation during the first 3 days (Fig. 2). The fact suggests the possibility that the potentiality of conidial formation on conidiophore was inhibited by near-UV, during the conidial development stage as suggested in *Alternaria dauci*²³⁾ and *Helminthosporium teres*¹⁷⁾. Therefore, it is presumed that the accelerated process and increased amount of conidia in sporulation of *P. oryzae* might be mainly due to the stimulatory effect of near-UV in the formation of conidiophore.

It is well known that abundant spores of *P. oryzae* were released in the midnight not only in the field^{8,18)} but also in the laboratory when blast lesions on the neck were provided with alternating light-darkness¹⁹⁾. With an isolate of *Helminthosporium oryzae* HA₂, Honda *et al.*⁵⁾ also found that maximum sporulation occurred on potato dextrose agar under continuous near UV irradiation followed by a period of darkness within 24 hr and suppressed by exposure to blue light after inductive period. These results might well explain why more the conidia could be trapped in the midnight than those in daytime in nature. Since the both fungi being exposed to solar radiation on a diurnal cycle in contrast to the continuous exposures used in this study, processes of sporulation by *P. oryzae* with alternating light-darkness would be worthwhile to study further for better understanding epidemiology of blast disease in nature.

The presence of photoreceptors or sporogenic substances^{11,21)} in connection with photoactivation process^{12,22)}, or photo-inhibition process^{13,14)} has been assumed. Even if sporulation of *P. oryzae* does not necessarily require light, the fact that near-UV stimulates sporulation process indicates that there may be a photochemical process and photoactivated sporogenic substances.

Spectroscopic analysis of fungal extracts of *P. oryzae* showed that water soluble substances absorbing near-UV radiation in the region of wave length between 300 and 360 nm (Fig. 3) were produced in irradiated colonies but not found in unexposed colonies grown for 4 days. This absorption region is similar to the wave length effective in inducing sporulation¹⁶⁾. Furthermore, the biological assay with fungal extracts supports the hypothesis that such substances may be activated the sporulation by irradiation with near-UV. Therefore, these heat-unstable substances, of which production was promoted also by near-UV, might be presumed as the kind of photoreceptor associated with photoactivated sporulation of *P. oryzae*.

On the contrary to the experiments of Trione *et al.*²¹⁾, however, colonies grown on the media containing these fungal extracts in the dark showed less sporulation than the control. Thus it was not appeared that the near-UV absorbing substances acted directly on sporulation or were sporogenic by themselves.

On the other hand, it is interesting that chlorogenic acid⁶⁾ known to have almost same absorption spectrum⁶⁾ as that of fungal extract detected in this study⁹⁾ stimulated the conidiophore formation of *P. oryzae* in light, but in the dark had no effect on elongation of conidiophore⁷⁾.

The present study shows that the accelerated and increased sporulation of *P. oryzae* in near-UV irradiation is mainly due to the conidiophore formation stimulated through the activation of the near-UV absorbing substances in fungal colony, its production is also promoted by near-UV. It may also be concluded that the effect of near-UV irradiation on sporulation depends upon many factors that may vary with isolates and with the phase of sporulation.

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和文摘要

イネいもち病菌の分生孢子形成におよぼす近紫外線照射の影響

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イネいもち病菌の分生孢子形成におよぼす近紫外線照射の影響を知るため、ジャガイモ蔗糖寒天培地全面に孢子、菌糸懸濁液を接種し、 $27\text{C}\pm 1$ において形成される孢子量を日を追って測定した。一般に近紫外線 (366 nm) の連続的照射は供試した4菌株を通じて孢子形成量の最高になるまでの日数を短縮したが形成される孢子の量は菌株によってちがった。はじめの3日間の連続照射は孢子形成量を最高にし、孢子柄の形成を促進した。連続暗黒下または初めの3日間の暗黒下では孢子形成量は劣った。水溶性、熱不安定、325 nm に最大吸収をもつ物質が4日間照明した培養の菌叢から抽出されたが暗黒下で培養したものからはえられなかった。この抽出物をジャガイモ蔗糖寒天培地に添加すると近紫外線照射下では顕著に孢子形成を促進したが、高圧加熱した抽出物はその作用を失う。この抽出物を加えて暗黒下においた場合は孢子形成は抑制された。抽出物を高圧加熱しても同様であった。