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Short Communication

Suppression and reactivation of UV-induced sporulation by blue light in *Bipolaris oryzae*

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Sporulation in *Bipolaris oryzae* was induced by UV radiation (295 nm), but the number of conidia gradually decreased with increasing duration of UV radiation longer than 1 min. The inductive effect of UV radiation can be nullified by blue light (459 nm) applied immediately before or after inductive UV radiation shorter than 1 min. In contrast, the number of conidia increased with an increasing duration of blue light applied after inductive UV radiation longer than 1 min, but not if it was applied before UV radiation. The present study firstly revealed the possibility of photoreactivation in *B. oryzae* sporulation.

Key Words——*Bipolaris oryzae*; blue light; reactivation; sporulation; UV radiation.

Near-ultraviolet radiation (NUV; 300–400 nm) and blue light (400–500 nm) induce various responses in fungi. It has been reported that sporulation in certain fungi imperfecti such as *Bipolaris oryzae* (Breda de Haan) Shoem., *Alternaria tomato* (Cook) Weber, *A. cichorii* Nattras, and *Botrytis cinerea* Pers.:Fr. was controlled by an antagonistic action of blue light and NUV radiation mediated through a 'mycochrome' system at two developmental stages: induction of conidiophore formation and conidiophore maturation (Honda et al., 1968; Kumagai, 1978, 1988). Conidiophore formation in *B. oryzae* is induced by NUV radiation, and conidia develop in the subsequent darkness. However, the inductive effect of NUV radiation can be nullified by blue light applied immediately after or before inductive NUV radiation. When conidiophores are exposed to blue light at a certain conidiophore maturation stage, they dedifferentiate into sterile conidiophores, and conidia do not form. However, the inhibitory effect of blue light on sporulation can be nullified by NUV radiation applied immediately after blue light. The effects of blue light and NUV radiation are reversible, and the final response depends on the last type of radiation administered (Honda et al., 1968). Isolates of *B. oryzae* with a 'mycochrome' system that controls conidiophore formation through the antagonistic action of blue light and NUV radiation were found to be widely distributed in paddy fields in Japan (Kihara et al., 1997).

On the other hand, the phenomenon of photoreactivation (the reduction of lethal and mutagenic effects of UV radiation by simultaneous or subsequent irradiation with blue/UV-A light) has been identified in such fungi as *Aspergillus* (Mishra and Bhattacharya, 1975), *Trichoderma* (Sametz-Baron et al., 1997), and *Phycomyces* (Galland, 1996). In enzymatic photoreactivation, UV-induced pyrimidine dimers in DNA are converted into the constitutent pyrimidines using photon energy (Hearst, 1995) by a photoreactivating enzyme, photolyase, with the help of blue/UV-A light. The systematic study of this type of DNA repair started with the discovery of photorecovery in conidia of *Streptomyces* (Kelner, 1949). The recent recognition that a putative blue light-receptor gene in *Arabidopsis thaliana* (L). Heynh. (Ahmad and Cashmore, 1993; Hoffman et al., 1996), *Sinapis alba* L. (Malhotra et al., 1995) and *Chlamydomonas reinhardtii* Dangeard (Small et al., 1995) shows substantial sequence homology with prokaryotic and eukaryotic DNA photolyase has made these DNA repair enzymes relevant models for blue/UV-A photoreceptors. Because of the close evolutionary relation between DNA photolyase and blue/UV-A photoreceptors, it is of interest to investigate photoreactivation and photomorphogenesis in photo-controlled sporulation in the same organism. However, photoreactivation in *B. oryzae* has not been investigated. The present study aimed to reveal the photoreactivation in the UV induced conidiophore formation controlled by an antagonistic action of blue light and UV radiation mediated through the 'mycochrome' system in *B. oryzae*.

An experimental strain of *B. oryzae* Bo.3 (formerly *Helminthosporium oryzae* Breda de Haan), isolated from an early blight lesion of a rice leaf from an experimental field of Shimane University, was grown on V-8 agar medium (Campbell's V-8 juice 200 ml, $CaCO_3$ 2 g, agar 17 g, distilled water 800 ml, with an initial pH of 5.8; 10 ml/60 mm diam. Pyrex Petri dish) at 25±1°C. Colonies grown for 4 d in the dark were treated with UV radiation (295 nm) to induce sporulation and/or blue light (459 nm) to inhibit sporulation, because action spectra

for induction and inhibition of sporulation in *B. oryzae* showed peaks at 298 nm and 447 nm, respectively (Yamamura et al., 1977; Kumagai, 1983). During irradiation, the cover lids of the Petri dishes were removed. After 24 h of darkness following the irradiation, the colonies were flooded with 99% ethanol and covered with glass covers, and conidia were microscopically (× 100) counted in several areas (each 1 mm^2) within the irradiated area of each colony. The results of replicated experiments and replications were averaged. Statistical analysis was carried out with the computer program Super Anova (Abacus Concepts Inc., Berkeley, Ca. USA). The UV radiation of 295 nm for induction and blue light of 459 nm for inhibition of conidiophore formation were obtained from a grating monochromator (CT-10; Japan Spectroscopic, Co., Tokyo, Japan). The light was introduced vertically from the top using a surface mirror by the method of Kumagai (1982). The irradiance of UV radiation and blue light was measured using a thermopile with a quartz window (MIR-100Q; Mitsubishi Yuka, Yokkaichi, Japan) coupled with a digital multimeter (TR6846; Advantest, Tokyo, Japan) at 323 and 810 $\mu W\ cm^{-2}$, respectively.

When dark-grown colonies were exposed to UV radiation, conidiophore formation was induced and conidia developed in subsequent darkness. As shown in Fig. 1, the number of conidia induced by UV radiation increased with short lag, then increased steeply as the irradiation increased. However, the number of conidia gradually decreased with increasing duration of irradiation longer

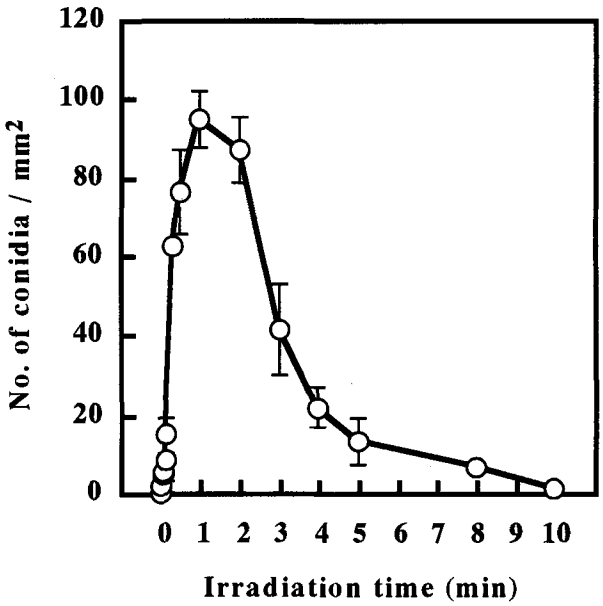

Fig. 1. Sporulation induced by irradiation with UV radiation at 295 nm.
Colonies grown for 4 d in the dark were exposed to UV radiation. After 24 h of darkness following the inductive irradiation, the number of conidia was counted in several areas (each 1 mm^2) within the irradiated area of each colony. Vertical bars show standard deviations of three replicate experiments.

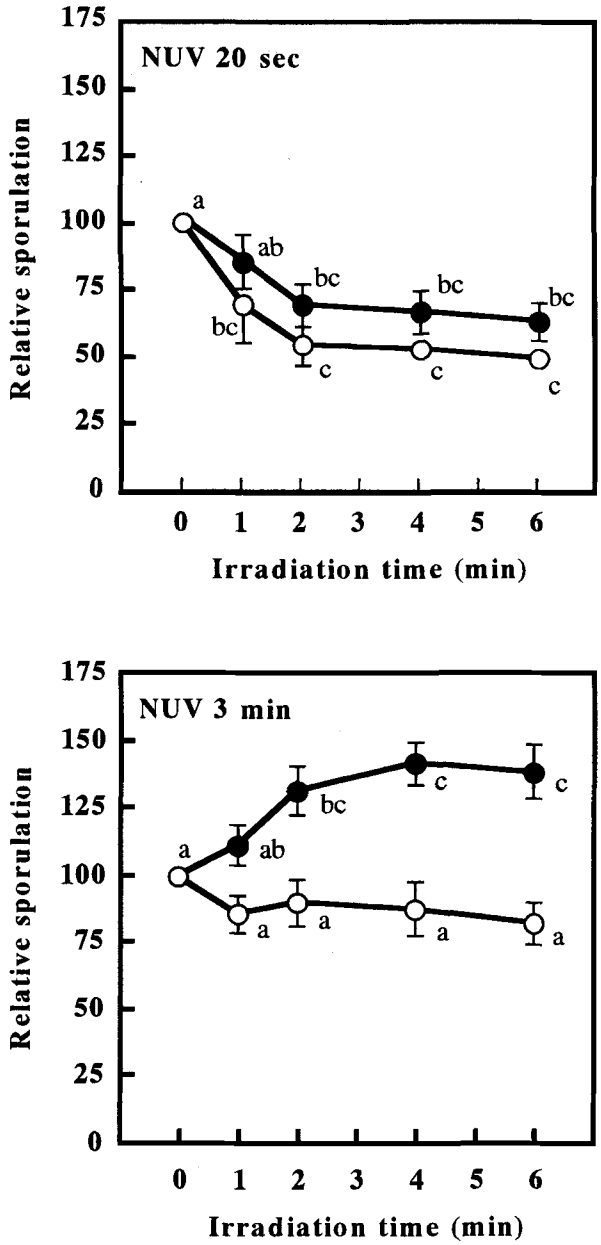

Fig. 2. Effect of pre- (open circle) and post- (closed circle) irradiation with blue light at 447 nm on sporulation induced by irradiation with UV radiation at 295 nm for 20 sec and 3 min.
Colonies grown for 4 d in the dark were exposed to blue light applied immediately before or after inductive UV radiation. After 24 h of darkness following the inductive irradiation, the number of conidia was counted in several areas (each 1 mm^2) within the irradiated area of each colony and are expressed relative to that of conidia without exposure to blue light. Vertical bars show standard deviations of three replicate experiments. Values followed by the same letter are not significantly different at $p=0.05$ according to Duncan's new multiple-range test.

than 1 min. These results suggest that UV radiation has two effects on sporulation in *B. oryzae*: induction and inhibition of sporulation. It has been reported that UV radiation induces not only photomorphogenesis but also DNA damage such as pyrimidine dimer formation. We therefore examined whether the decrease of sporulation under prolonged irradiation of UV radiation was caused by DNA damage.

The inductive effect of NUV radiation can be nullified by blue light applied immediately before or after inductive NUV radiation in *Alternaria tomato*, in which sporulation is controlled by the antagonistic action of blue light and NUV radiation mediated through the 'mycochrome' system, as in *B. oryzae* (Kumagai, 1982, 1985). Fig. 2 shows the effect of blue light applied immediately before or after inductive UV radiation. When colonies were exposed to blue light before or after inductive UV radiation for 20 sec, the number of conidia decreased with increasing duration of blue light. In contrast, the number of conidia increased with increasing duration of blue light when colonies were exposed to blue light after inductive UV radiation for 3 min. The increase of conidia by blue light after UV radiation was observed after an excessive dose of inductive UV radiation. Furthermore, the sporulation recovered if blue light was applied immediately after UV radiation, but not if it was applied before UV radiation. These results suggest that DNA damage such as UV-induced cyclobutane pyrimidine dimers (CPDs) caused by excessive UV radiation, which has an inductive effect on sporulation, would be photoreactivated by blue light, which has specific effect of inhibition of sporulation. When dark-grown colonies were exposed to UV radiation for 3 min, about 40 conidia per 1 mm^2 were formed in the subsequent darkness (Fig. 1). When colonies were exposed to blue light after inductive UV radiation for 3 min, the number of conidia increased about 1.5-fold (about 60 conidia per 1 mm^2) as compared with the control colonies without blue light irradiation (Fig. 2). However, number of conidia did not increase to the maximum of about 90 conidia per 1 mm^2 induced by the UV radiation for 1 min. It was speculated that the decrease of sporulation might be caused by other, irreversible reactions.

Photoreactivation phenomena in fungi have been observed mainly in relation to the survival of the spores under UV radiation (Mishra and Bhattacharhy, 1975; Sametz-Baron et al. 1997). The present study revealed the possibility of photoreactivation in sporulation as one stage of photomorphogenesis. It was presumed that the effects of UV (induction of sporulation and DNA damage) and those effects of blue light (inhibition of sporulation and photoreactivation) would interact in a complex manner in the process of light perception to gene expression for sporulation in *B. oryzae*.

This study suggested that *B. oryzae* may have a photoreactivation system. Photoreactivation in the filamentous fungus *B. oryzae* is of interest because its blue/UV-A photoreceptors (cryptochromes) may share homology with DNA photolyase. A DNA photolyase gene, *phr*, in fungi was isolated from *Saccharomyces cerevisiae* Hansen (Sancar, 1985) and *Neurospora crassa* Shear et Dodge (Yajima et al., 1991). Cloning and analysis of *B. oryzae* photolyase gene should increase our understanding of the blue/UV-A photoreceptor in sporulation controlled by an antagonistic action of blue/UV-A and NUV radiation mediated through the 'mycochrome' system.

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