SHORT COMMUNICATION

Gamma-ray Induced Delay of Fruiting Body Initiation in a Basidiomycete, *Hebeloma vinosophyllum*

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The effect of gamma-radiation on fruiting body initiation in a basidiomycete, *Hebeloma vinosophyllum*, was investigated. Fruiting of this fungus is induced by visible light, but irradiation of the mycelium before or after light treatment delayed fruiting body initiation. The time required for fruiting body initiation increased with the radiation dose. The induction of fruiting bodies had two gamma-radiation sensitive stages, one immediately before fruiting body initiation and the other 15 to 20 h after the start of photoinduction.

INTRODUCTION

Initiation of basidiomycete fruiting bodies from vegetative mycelia is controlled by environmental, nutritional and genetic factors (Kitamoto *et al.*, 1975; Suzuki, 1979; Uno & Ishikawa, 1971). In some species there is a light requirement for fruiting body initiation (Durand, 1976; Robbins & Hervey, 1960).

Ionizing radiation normally inhibits biological processes (see, for example, Bacq & Alexander, 1961). Radiation effects on nuclear division of slime moulds have been described (Matsumoto & Sawada, 1978; Sachsenmaier *et al.*, 1970), but little is known of the effect of radiation on morphogenesis of higher fungi other than reports of its effect on fungal spores (Assche *et al.*, 1977; Pomper & Atwood, 1955).

The fungus *Hebeloma vinosophyllum* is readily cultured and its fruiting body initiation is induced by light. In this study it was found that gamma-irradiation delays the fruiting body initiation and that two radiation-sensitive stages occur during the process.

METHODS

Fungus. The basidiomycete *Hebeloma vinosophyllum* Hongo was kindly given by M. Aoki (Tokyo Metropolitan Research Laboratory of Public Health). The development of its fruiting body is divided into several stages (Suzuki, 1976). Abundant aerial hyphae are first produced on a mycelial colony, followed by the production of primordia which form a pale brown tight cluster 1 mm high. In this study, fruiting body initiation refers to the production of primordia.

Culture conditions and treatment. Mycelium was grown on an agar medium consisting of 10 g malt extract (Difco), 2 g yeast extract (Difco), 15 g agar (Wako Pure Chemical Industries, Osaka, Japan) and 1 l distilled water. The medium was adjusted to pH 5·8 with HCl and sterilized by autoclaving for 15 min at 121 °C. Mycelium was grown on 18 ml medium in 9 cm diam. Petri dishes at 25 °C. A mycelial inoculum consisted of a 4 mm diam. agar disc taken from the peripheral region of a mycelial colony which had been grown for 7 d in darkness. For growth in the dark, dishes were wrapped in black paper and aluminium foil. A white fluorescent lamp (10 W, Matsushita Electric Industries, Tokyo, Japan) was used as the light source to initiate fruiting; the light intensity was 200 to 400 $\mu W\ cm^{-2}$ at the surface of the dish. A cobalt-60 irradiation facility (Kimura Kako Co., Osaka, Japan) was used to irradiate the cultures at a dose rate of 1 krad min^{-1}. Samples were automatically carried to the ^{60}Co source in a cylindrical drawer (16 cm diam.). Five dishes, in

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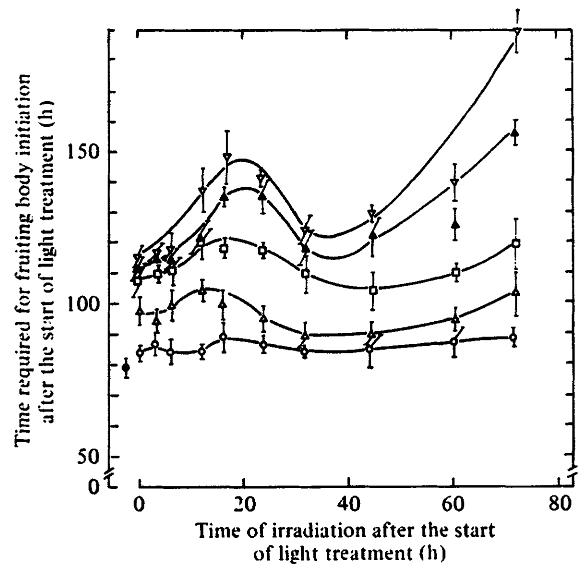

Fig. 1. Gamma-ray induced delay of fruiting body initiation. Mycelia grown for 7 d in darkness were irradiated at different doses and at different times after the start of light treatment: ○, 0·5 krad; △, 1 krad; □, 2 krad; ▲, 3 krad; ▽, 5 krad; ●, unirradiated. Each point represents the mean of results for 10 dishes; vertical bars indicate standard errors.

a pile, were irradiated simultaneously. Readings taken with a Fricke dosimeter confirmed that the same dose was given to each dish.

RESULTS AND DISCUSSION

The time required for fruiting body initiation after the start of light treatment decreased with increase in the period of growth in darkness after inoculation. However, no further decrease was observed when mycelium had been grown in darkness for 7 d or longer and under these conditions primordia were produced approximately 80 h after the start of light treatment.

To investigate the possible inhibition of mycelial growth by gamma-irradiation, 7 d dark-grown cultures were irradiated and then incubated for 5 d in the light at 25 °C before colony diameters were measured. No detectable inhibition of mycelial growth was observed at 30 krad, but higher doses markedly reduced mycelial growth and little or no growth was observed at doses above 90 krad. In subsequent experiments on fruiting body initiation, a dose of less than 5 krad was used since this did not affect mycelial growth.

The effect of gamma-irradiation on the time required for fruiting body initiation was then investigated. Mycelia which had been grown for 7 d in darkness were irradiated (1 krad) and then incubated in darkness for different periods before light treatment was started. Irradiation delayed fruiting body initiation by 20 h, but a 6 h dark period after irradiation reduced the delay to 10 h, and there was no delay if dark treatment lasted for 12 h or more. Irradiation did not reduce the number of primordia produced. The time required for fruiting body initiation was also determined when cultures were irradiated at different doses after the start of light treatment (Fig. 1). Only limited inhibition of fruiting body initiation was observed at 0·5 krad. At higher doses, fruiting body initiation was significantly delayed. When cultures were irradiated at 5 krad at the same time as the start of light treatment, it was 115 h before primordia were produced, whereas on unirradiated mycelia primordia were produced about 80 h after the start of light treatment. Although mycelia irradiated at 5 krad produced fewer primordia than unirradiated mycelia, at the lower doses the number of primordia was not significantly reduced indicating that the process of fruiting body initiation was reversibly inhibited by irradiation. The time required for fruiting body initiation in mycelia irradiated at different times after the start of light treatment is also shown in Fig. 1. At all doses used, mycelium irradiated at either 15 to 20 h or 72 h after the start of light treatment required longer to produce primordia than mycelium irradiated at other times. It was concluded that there are two radiation-sensitive stages between the beginning of photoinduction and primordia formation.

Ionizing radiation can retard mitosis leading to a delay or cessation of cell division (Bacq & Alexander, 1961; Matsumoto & Sawada, 1978; Sachsenmaier *et al.*, 1970). The observed delay in primordia production caused by gamma-irradiation may be due to delayed nuclear division resulting in delayed cell division. Alternatively, biochemical processes associated with fruiting body initiation may be damaged by radiation. In this case, delay in primordia production would be due to the repair or reconstitution of this process, which is light-mediated. The first of the two radiation-sensitive stages found in the present study may correspond to the initiation of biochemical processes which are light-induced and which initiate fruiting body formation. The second stage occurs immediately before primordia production, corresponding to the active growth of aerial hyphae. This is a process of rapid cell division which may be readily affected by radiation. The delay in fruiting body initiation in the mycelia irradiated at the second stage may therefore be due to inhibition of cell division by radiation.

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