Phototropism in Yeast: A New Phenomenon to Explore Blue Light-Induced Responses

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Although yeasts have been intensively investigated in photobiology, directional response of yeast growth to light has never been observed. The present data demonstrate for the first time phototropism in yeast, the basidiomycetous yeast *Sporobolomyces salmonicolor.* The effective spectral band is blue light – suggesting that a blue-light receptor similar to that in other plants is involved in yeast photophysiology. Further studies on yeast phototropism could help identification of the photoreceptor and throw new light on the mechanisms of signal transduction and response.

Introduction

Growth and development in plants is profoundly affected by blue light (Briggs, 1993; Senger and Schmidt, 1994). The diverse effects triggered by blue light are generally classified as metabolic, morphological and directional. The physiological significance of these effects challenges many researchers to unravel the identity of the photoreceptors and the signal transduction mechanisms causing the response (Ahmad and Cashmore, 1993; Quinones and Zeiger, 1994).

Among the most intensively studied responses to light is phototropism – orientation of a growing part of a plant toward or away from light. The phenomenon is typical in many green plants and widely known in lower plants including fungi as well (Firn, 1994). The phototropic response is initiated after detection of light intensity spatial gradient by photoreceptors, in most cases not identified.

Investigations on light effects in yeast have been performed covering the whole range of visible and ultraviolet spectra (Woodward *et al.,* 1978; Ulaszewski *et al.,* 1979; Edmunds, 1980; Quickenden *et al.,* 1989; Tada *et al.,* 1990; Gil-Hwan and Johnson, 1990), but to our knowledge the above mentioned studies involve only symmetrical, uniform irradiation and there are no data on phototropic response in yeast under unilateral irradiation.

The present study shows for the first time phototropism in yeast, using the basidiomycetous *Sporobolomyces.* The finding of a new organism displaying phototropic response opens the way for comparative investigations based on the information available for other fungi and green plants aiming elucidation of photoperception – signal transduction – response systems.

Materials and Methods

The strain *Sporobolomyces salmonicolor* AKU 4428 used in this study was a kind gift of Prof. Sakayu Shimizu (Lab. Fermentation Physiology, Agricultural Department, Kyoto University, Kyoto, Japan). It originates from *Sporobolomyces salmonicolor* IFO 0375 (Collection of the Institute of Fermentation, Osaka, Japan). Even though we found some slight morphological differences between these two strains, light elicited phototropic response in strain IFO 0375 as well. *Sporobolomyces salmonicolor* AKU 4428 was maintained on agar slants with the medium composed of 5 g/l yeast extract (Difco Lab., Detroit, U.S.A.), 10 g/l bactopeptone (Difco), 10 g/l dextrose (Nacalai Tesque, Inc., Kyoto, Japan), 20 g/l agar (Koso Chemical Co., Ltd., Tokyo, Japan). Due to the highly branched, sticky mycelial structure of the colonies grown on solid medium it was more convenient for inoculation to use liquid culture. The

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liquid culture was performed with the same medium as stated above without agar, at 28 °C, on a rotary shaker in the dark.

In the morphogenesis experiments the cultures were incubated for 3 days at 20 °C on agar plates with 50 g/l malt extract (Difco), 3 g/l yeast extract (Difco), 20 g/l agar (Koso Chemical Co., Ltd.).

In the experiments with unilateral irradiation inoculation was performed by dipping sterile, microscope cover slips (18×18 mm) coated with solid growth medium into diluted yeast suspension. The medium was designed to ensure nutrient deprivation resulting in formation of flat colonies with extended surface hyphae. The medium consisted of 5 g/l Yeast Nitrogen Base (Difco), 1 g/l dextrose (Nacalai Tesque, Inc.), 20 g/l agar (Koso Chemical Co., Ltd.). The concentration of yeast suspension was adjusted to allow the growth of individual colonies on the cover slip surfaces or edges (Fig. 1). The cover slips were placed vertically in transparent containers by inserting them partially into agar medium. The containers were placed laterally into black boxes. The cover slips were adjusted parallel to the incident light: in Fig. 2 the *x*-axis is parallel to the light beam.

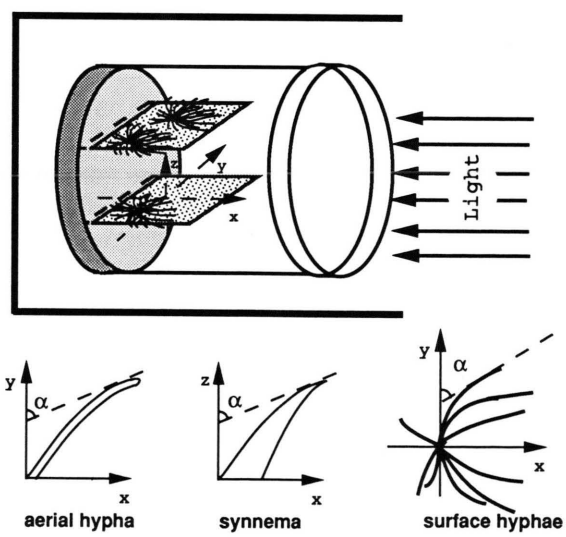

Fig. 1. Scheme of the experimental set up and bending angle determination used in the estimation of phototropism. Yeast was inoculated on microscope cover slips coated with medium under conditions favoring spreading pattern of colony growth and subjected to unilateral irradiation.

Irradiation conditions were as follows: The light from a white fluorescent lamp (cool white, Toshiba FL 40 SD/38, Toshiba, Japan) was passed through heat-absorbing filters. Irradiation was initiated immediately after yeast inoculation. Equilateral irradiation was delivered from above. Unilateral irradiation was achieved by using black boxes (13×21×13 cm) with a window left open in the case of white light and UV-A light or closed by plexiglas filter (Rohm and Haas, Philadelphia, U.S.A.) where the light passed through. In the case of blue light irradiation a broad-band pass filter #2264 with transmittance peak at 453 nm and half-band width 132 nm was used. For the red light the filter was #2444, cutting the light with wavelength shorter than 584 nm. UV-A light was obtained from a 20 W black light lamp (FL 203 BLB lamp, Toshiba) with maximum emission at 360 nm. Dark controls were grown in closed black boxes.

Light intensities were measured at the level of the irradiated samples with a calibrated custom-made thermopile radiometer (thermopile modules MIR 100 Q, Mitsubishi Yuka, Tokyo, Japan).

The curvature of hyphae and synnemata [synnema (pl. synnemata) is a group of hyphae joined together], due to phototropic response was measured on photomicrographs after appropriate magnification. The deviation of the filaments from the corresponding axis was determined as shown in Fig. 1. Where surface hyphae were considered, the curvature was estimated based on the deviation of the hyphal tip from the colony diameter drawn on the photoprints perpendicularly to the incident light. It is evident that the maximum theoretical response is proportional to the initial angle between the hypha and the incident beam. Since some shading effects could prevent correct estimations in the sector 90°–270° (angle 0° means parallel to the incident light) we have chosen to consider only hyphae in the vicinity (within 10°) of the diameter perpendicular to the incident light. The close correlation between the bending angle found in surface hyphae using this method and the bending angle measured in aerial hyphae proves the validity of our approach. In the case of dark control the measurements were taken in the vicinity of the diameter parallel to the closed window.

For scanning electron microscopy samples have been fixed with 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) followed by fixation with

1.5% osmium tetroxide in cacodylate buffer. After dehydration through a graded ethanol series and critical point drying in liquid carbon dioxide, the samples have been sputter coated with palladium.

Results and Discussion

Experiments were performed with *Sporobolomyces salmonicolor* AKU 4428. The reason to choose this particular strain was its morphology with predominating hyphae which is an advantage when directional response is sought. The surface hyphae often protruded single hyphae in the air. Light-grown colonies exhibited bright salmon color and formation of specific structures causing a rough appearance of the colony surface (Fig. 2A). Scanning electron microscopy revealed that these structures comprise many hyphae stuck together in parallel (Fig. 2C), and we designated them as "synnemata" – a term generally used for similar structures in other lower fungi. Dark-grown colonies were less pigmented, with smooth and shiny surface (Fig. 2B), without synnemata (Fig. 2D) at least during the first three days of incubation. In older cultures synnemata were formed in the dark, but obviously few and less developed as compared with these of the light-grown colonies.

When exposed to unilateral irradiation (Fig. 1), a positive phototropism was exhibited in all three kinds of filaments: well-developed aerial synnemata (Fig. 3A–B), aerial single hyphae (Fig. 3C) and surface hyphae (Fig. 4). Usually the response was obvious in colonies grown more than three days suggesting dependency on the developmental stage. When the light direction was switched and already bent synnemata were subjected to light coming from the opposite side, we observed initiation of new synnemata stemming from the primary ones and growing toward the newly estab-

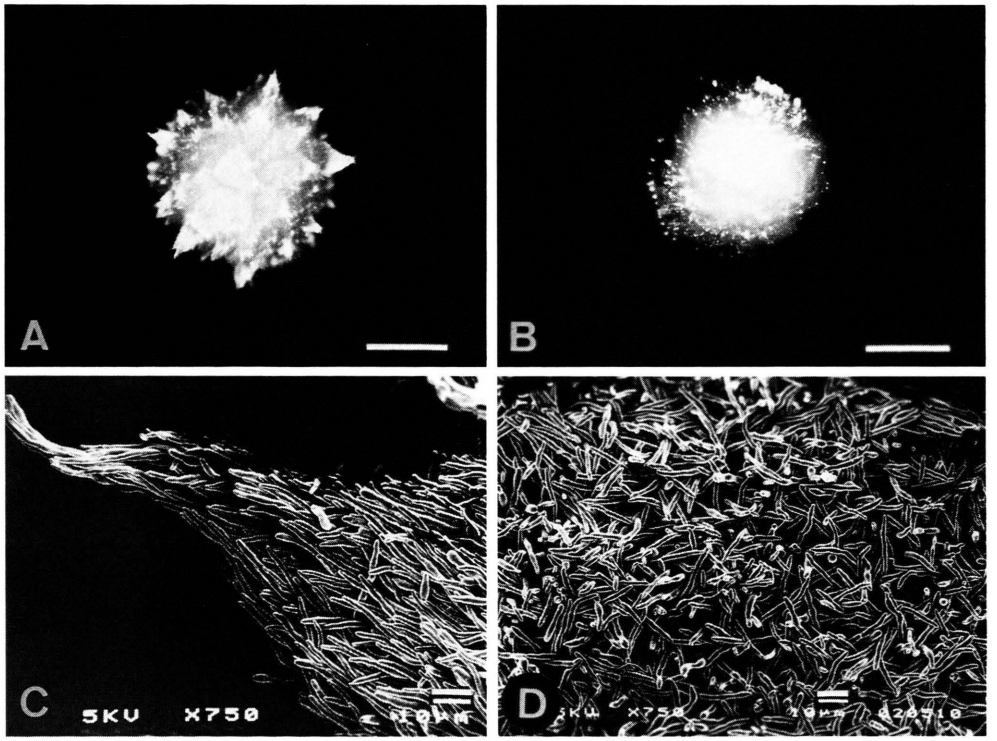

Fig. 2. Morphogenesis in colonies of *Sporobolomyces salmonicolor* AKU 4428 grown under different light conditions. A–B) Stereomicroscopic photographs. Light-grown colony under overhead white fluorescent light at the intensity of 1 W/m^2 (A) and control dark-grown colony (B). The scale bars represent 0.5 mm. C–D) SEM micrographs. Light-grown colony with the fine structure of a synnema (C) and random-scattered hyphae on the surface of dark-grown colony (D). The scale bars represent 10 μm.

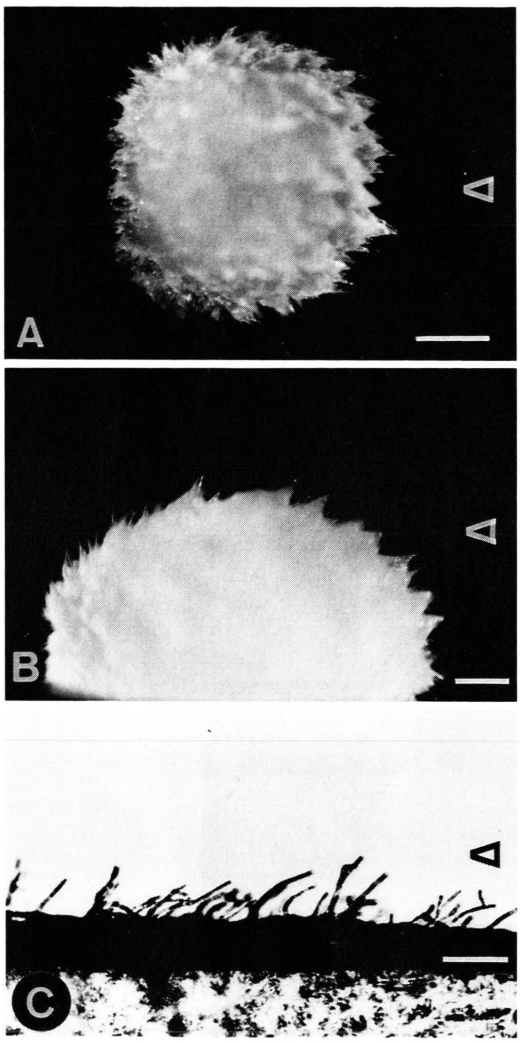

Fig. 3. Positive phototropism in *Sporobolomyces salmonicolor.* A–B) Orientation of synnemata toward the incident white light coming from right side (arrow-heads). Observation using stereomicroscope. Top view of the colony (A) and side view of the same colony (B). The scale bars represent 0.5 mm for (A) and 0.25 mm for (B). C) Orientation of aerial hyphae toward the light (arrow-head). Observation using transmission light microscopy. The scale bar represents 25 μm.

lished light direction. If still elongating, the tips of the primary synnemata bent toward the new direction (data not shown).

Experiments to check the active bands of the light spectrum showed that all hyphal structures are sensitive to blue and UV-A bands, but not to red light (Fig. 5). The statistical analysis of the bending data showed significant directional orientation in the samples irradiated with white, blue or UV-A light but not in red light sample or dark control.

The present results demonstrate unequivocally that *Sporobolomyces salmonicolor* belongs to the group of so-called blue light-sensitive organisms. The correlation in the phototropic response displayed by the different filaments (surface hyphae, aerial single hyphae and synnemata) of the culture suggests that there is no principal difference in the photoreceptor and signal transduction mechanisms involved in their bending.

In lower fungi the model system widely used for studies in phototropism is *Phycomyces* (Galland and Lipson, 1987; Ootaki *et al.,* 1988, 1991). However, because of the sensitivity to gravity, in experiments on photoinduced directional responses in *Phycomyces* the interference between both stimuli (gravity and light) has to be considered (Firn, 1990, 1994). We performed experiments with inverted, non-inverted and vertically positioned agar plates in the dark, using different media and temperatures. There was no evidence in any of the filaments produced for bending or length change in response to gravity (data not shown). The lack of gravitropic behavior in *Sporobolomyces* is an advantage since "single stimulus" experiments can be easily performed.

Phototropic behavior in yeast may not be inherent only to *Sporobolomyces salmonicolor.* The strategy for search of strains competent in phototropism can be designed using the knowledge on the response mechanism in other plants and fungi. Thus, in many cases differential growth underlying phototropic response is achieved by light-induced growth inhibition (Elliott and Shen-Miller, 1976; Firn, 1990). Since the growth of many yeasts *(e.g. Saccharomyces, Candida, Rhodotorula, Pfaffia)* has been shown to be inhibited by light (Woodward *et al.,* 1978; Ulaszewski *et al.,* 1979; Edmunds, 1980; Quickenden *et al.,* 1989; Gil-Hwan and Johnson, 1990), it is plausible that under certain conditions such photosensitive strains show phototropic response.

The present study is the first report on phototropism in yeast. Together with the findings that the dimorphic yeast *Candida albicans* can show thigmotropism (Sherwood *et al.,* 1992) and electrotropism (Crombie *et al.,* 1990), the phototropism shown here is a new evidence supporting the ap-

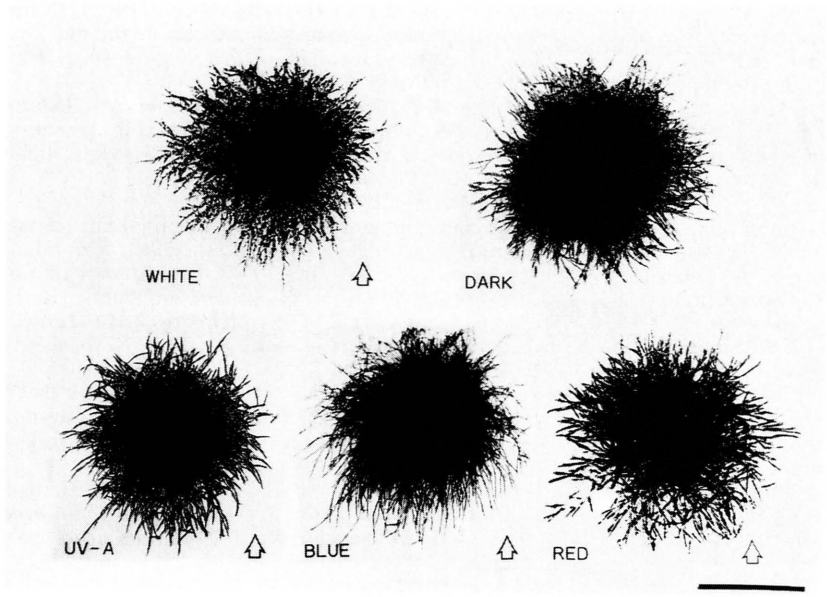

Fig. 4. Phototropism in surface hyphae of *Sporobolomyces salmonicolor* as influenced by the region of light spectrum. Arrows show the direction of the light. White light, UV-A and blue light are effective for hyphal orientation. The scale bar represents 0.5 mm.

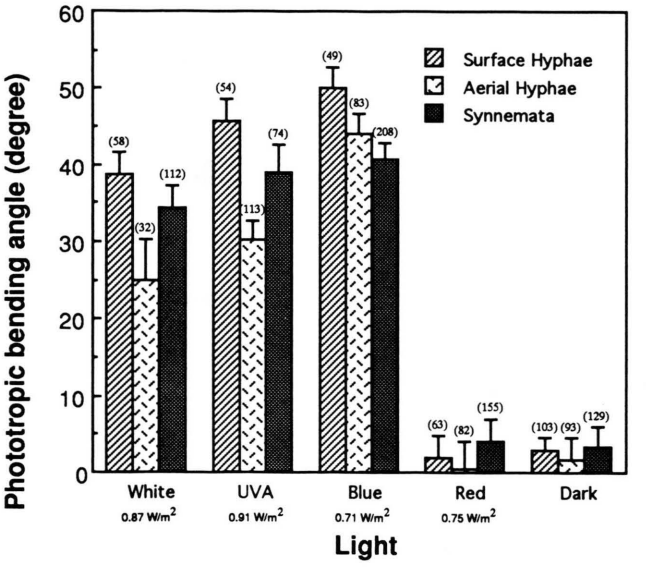

Fig. 5. Phototropic bending angle of hyphae and synnemata of *Sporobolomyces salmonicolor* in dependency of the light band. Numerals in brackets are numbers of filaments measured. Bars represent the standard errors.

plicability of yeasts in studies on the receptor(s) and signal transduction mechanisms of growth guided by external factors.

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