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ORIGINAL PAPER

Effect of light on growth, pigment production and culture morphology of *Monascus purpureus* in solid-state fermentation

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Abstract The capacity to sense and respond to light is widespread in animals, plants, fungi and bacteria. The effect of light quality on growth and pigment yield of *Monascus purpureus* was investigated. Incubation in total darkness increased red pigment production from 14. 5 OD/g dry substrate to 22 OD/g dry substrate. In contrast, growth of the fungus in direct illumination resulted in total suppression of pigment production. It was found that both red and blue light influenced pigment yield as well as culture morphology. The authors propose the existence of a light-perception system in *Monascus purpureus*.

Keywords Solid-state fermentation · *Monascus purpureus* · Red pigment · Direct illumination · Photoreceptors

Introduction

In most organisms light, like temperature, is a crucial environmental signal for regulating developmental and physiological processes. Consequently, the capacity to sense and respond to light is evolutionarily conserved throughout the kingdoms, from archaea and fungi to humans (Arden et al.1966). Electrical signals have been detected in response to light excitation of the fungus *Phycomyces blakesleeanus*. These signals are related to the wavelength and intensity of the stimulus and the growth stage of the fungus (Mogus and Volken 1974). The effects of light have been investigated in the model fungal species. While spectral analyses and the morphological effects of the light have been well characterized in genera such as *Coprinus* (a basidiomycete) or *Phycomyces* (a zygomycete), at the molecular level, *Neurospora crassa* (an ascomycete) is the best understood based on the functions of the *white collar* (*wc-1* and *wc-2*) genes in the light sensing (Kues 2000; Cerda-Olmedo 2001; Liu et al. 2003). Development of reproductive structures in a light-dependent manner has been reported in *A. nidulans*, promoting sexual development in the darkness while stimulating asexual sporulation under illumination (Bayram et al. 2008). Light-dependent accumulation of carotenoid pigments has been studied in Neurospora (Rau and Mitzka-Schnabel 1985). Photoinduced conidiation (asexual reproduction) of fungi provides an interesting model for biochemical, physiological, and morphological studies on differentiation (Lauter 1996). Casas-Flores et al. (2006) demonstrated the existence of an unprecedented cross talk between light and carbon sensing. New insights into the light transduction pathways in fungi have been revealed with the recent isolation of the *crgA* gene of *M. circinelloides* (Navarro et al. 2001). In addition, the recent completion of several fungal genomes has allowed the identification of fungal photoreceptor genes, many of them unexpected.

In response to increased consumer perception that natural colorants are safer, manufacturers have moved toward more natural and less synthetic colorants in food (Carvalho et al. 2003). Even for those synthetic colors which proved

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harmless in adequate levels, it still appears as "artificial color" on the food package – which is currently a bad advertising. This circumstance has inevitably increased demands for highly safe, naturally occurring edible coloring agents, one of which is pigments from the fungus *Monascus purpureus* (Lee 1995; Babitha et al. 2007). The solid-state fermentation of rice by *Monascus* has a long tradition in East Asian countries, which dates back at least to the first century (Pandey 1994). *Monascus* pigments are a group of secondary fungal metabolites called azaphilones, which have similar molecular structures as well as similar chemical properties (Francis 1987). Effect of light on mycelium development and spore formation of *Monascus* in shaking flasks has been reported by Miyake et al. (2005). However, little is known about the influence of light in solid cultures. The purpose of this study was to investigate the effect of light conditions on growth, pigment production and morphology by *Monascus purpureus* under solid-state fermentation.

Materials and methods

Culture

A culture of *Monascus purpureus* LPB 97 was used in the present study. It was maintained on yeast extract-peptone-glucose medium (YPG) (Hi-Media, Bombay); preserved at 4°C and sub-cultured once in every three weeks.

Inoculum preparation

Culture of *M. purpureus* LPB97 was grown on YPG slants. To fully sporulated (6–8 days old) agar slope culture, 10 ml of sterile distilled water was added. Then the spores were scrapped under strict aseptic conditions. The spore suspension obtained was used as the inoculum.

Solid-state fermentation

Jackfruit seeds used as substrate were obtained from locally. The seeds were cleaned and the white arils (seed coats) were peeled off. The seeds were sliced in to thin chips and tray dried and were ground in to powder. Five grams of jackfruit seed powder was taken into 250 ml Erlenmeyer flask and to this a salt solution (2 ml) containing (g/l) KH_2PO_4 2 g, NH_4NO_3 5 g, NaCl 1 g, $MgSO_4.7H_2O$ 1 g and distilled water was added to adjust the required moisture level. The contents of the flasks were mixed and autoclaved at 121°C for 20 min. The substrate was moistened with distilled water in such a way as to obtain final moisture content of 50%–60%. After thorough mixing, the wet substrates were autoclaved at 121°C for 20 min and cooled to room temperature. It was inoculated with the spore suspension containing 1.5×10^5 spores per ml of *Monascus purpureus* LPB97 and incubated at 30°C for 7 days.

To study the effect of different wavelengths of light on the growth and pigment production, the experiment was set-up based on the principle that a colored-glass paper allows only its particular color of light to pass through—it filters out the other colors of the spectrum (Fig. 1a). Fermentation flasks were wrapped in colored glass papers of red, blue and green and placed at equidistant (20 cm) from an illuminated light source (Philips CFL, 100W) and were kept inside the incubator. To study the effect of keeping under direct illumination, the flasks were directly kept under light source and for those to study the effect of keeping in total darkness, were covered with black paper (Fig. 1b). To study the effect of different wavelengths of light on the fungal morphology, YPG plate cultures inoculated with same number of spores were wrapped in the colored glass paper and kept under the same illuminated light source. The plate was partially covered with the black paper and exposed to illumination in order to find the impact of keeping under direct light and total darkness on the fungal morphology (Fig. 3).

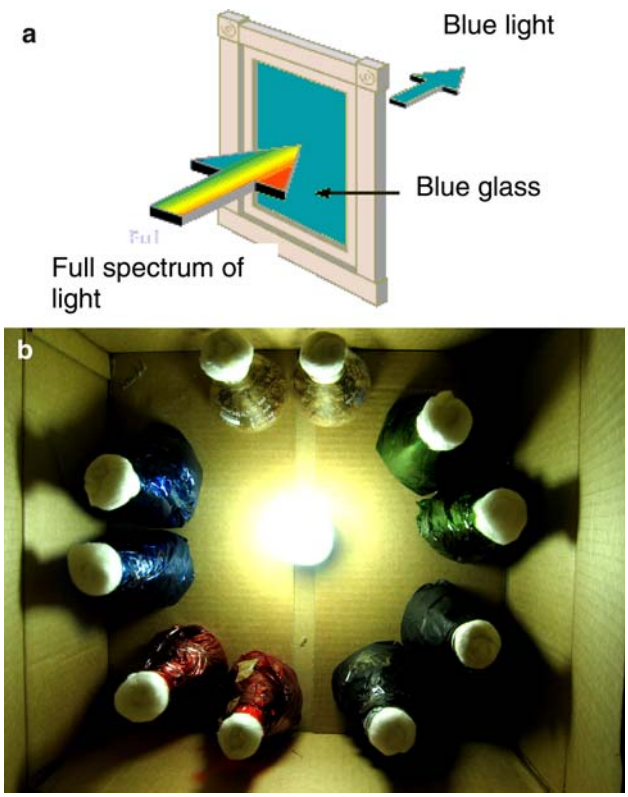

Fig. 1 (**a**) Principle, (**b**) Experimental set up to study the effect of light on pigment production and growth in *Monascus purpureus*

Pigment extraction and estimation

From the fermented solid substrate, a known amount was taken for pigment extraction using 90% ethanol. Five milliliters of solvent was added per gram of fermented material. The solvent and fermented sample was kept on a rotary shaker at 200 rpm for 1 h, allowed to stand for 15 min and filtered through Whatman#1 filter paper. Ethanol extract of unfermented sample was kept as the blank for pigment analysis so that any colored substances from the solid substrate were subtracted from the pigment produced by the fungus. The analysis of pigment production was done by measuring absorbance maxima (λ_{max}) of pigment extract by spectral analysis on the ranges near 400 and 500 nm for yellow and red pigments respectively (Lin et al. 1992) using a double beam spectrophotometer (Shimadzu, UV 1601) taking in to consideration the dilution factor of the sample (Chiu and Poon 1993). Pigment yield was expressed as optical density units per gram dry fermented matter multiplied by its dilution factor (Johns and Stuart 1991).

Biomass estimation

According to the definition of SSF, given by Durand et al. (1988) and Pandey (1994), microorganisms are intimately bound to the solid matrix, which involves difficulties for biomass measurements. So the fungal biomass estimation was carried out by determining the N-acetyl glucosamine released by the acid hydrolysis of the chitin, present in the cell wall of the fungi (Sakurai et al. 1977). Glucosamine released from the chitin by the acid hydrolysis was mixed with 1 ml acetyl acetone reagent and incubated in a boiling water bath for 20 min. After cooling ethanol (6 ml) was added followed by the addition of 1 ml of Ehrlich reagent and incubated at 65°C for 10 min. After cooling the optical density at 530 nm was taken against the reagent blank. Glucosamine (Sigma) was used as the standard.

Results and discussion

Effect of light in growth and pigment yield

In the fungal kingdom, light can regulate the growth, the direction of the growth, asexual and sexual reproduction, and pigment formation, all of which are important aspects for the survival and dissemination of the fungal species. Despite the importance of the light to the fungal development, much has yet to be determined to illuminate the mechanisms, the fungi use to perceive and respond to light. In this study, the absorption spectra of the pigments extracted from the light- and dark-grown mycelia indicated that pigment composition largely changes depending on the light conditions. Incubation in total darkness resulted in increased pigment production (about 2-fold). This observation finds significance as it is against the postulated photo-protective role of biopigments (Yong and Lee 1991; Salih et al. 2000; Seagle et al. 2005). In contrast, the growth of the fungus in the continuous illumination resulted in drastic reduction in the pigment yield. But when considering the growth of the organism, direct illumination favored the growth but under total darkness, there was a reduction in the biomass. Having shown that the light inhibits pigment production, the wavelength dependence of this response was investigated. Solid-state fermentation was carried out under different conditions of illumination, using the blue, green and red light. In all the three independent experiments, the red light showed little effect on the growth and pigment production. This result was in agreement with Miyake et al. (2005) where red pigment production in fermentation broth increased upon the red light exposure. On the contrary green and blue wavelengths inhibited the pigment production, even though there was an increase in biomass with green light (Fig. 2). Effect of blue light on metabolism, growth, sexual and asexual development, pigment formation, tropism and other phenomena has been studied in a wide variety of fungus. The effectiveness of blue light in inhibiting pigment production was not surprising. The vast majority of photoresponses, from the growth responses to phototropism, studied in the fungi are mediated by the photoreceptors that absorb blue light (Kumagai 1988, Lauter 1996).

Effect of different wavelength of light on culture morphology

The effects of different wavelength of light on the cultures were assessed by recording the visible phenotype observed in the YPG plates after 7 days of incubation. The colonies grown under total darkness showed profuse growth of aerial hyphae, giving a fluffy appearance to the colonies, and highly pigmented mycelia (Fig. 3b). When the plate was partially covered with the black paper and exposed to

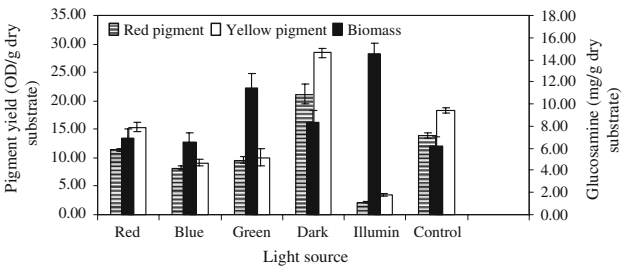

Fig. 2 Effect of different wavelengths of light, direct illumination and dark on pigment yield and growth of *Monascus purpureus*

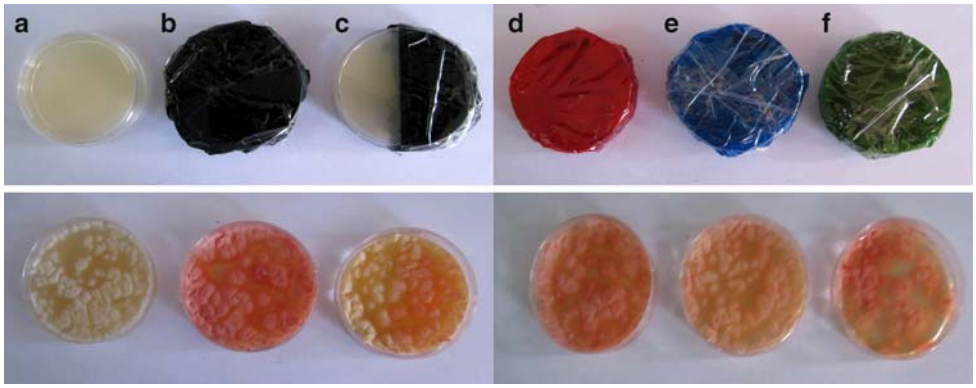

Fig. 3 Effect of different light sources on morphology of *Monascus purpureus* (**a**) direct illumination, (**b**) total darkness, (**c**) partially exposed to light, (**d**) red light, (**e**) blue light, (**f**) green light

illumination, the exposed region showed no pigmentation, whereas the covered portion showed pigmentation, thus, confirming the inhibitory role of illumination on the pigment production in *M. purpureus* (Fig. 3c). This observation suggested that the incubation in total darkness was most effective in inducing the pigment production. The colonies grown under the direct illumination showed no pigment production (Fig. 3a); this could be explained by postulating the existence of photoreceptors responsive to dark and light in this fungus. Under the red light, the colonies with profuse growth of aerial mycelium were formed (Fig. 3d). Similarly to the effect of white light, when the blue light was used, reduction in the colony size was observed (Fig. 3e). When green light was used, the flat colonies formed showed no significant differences in the appearance from those grown under the blue light (Fig. 3f).

The members of the fungal kingdom, which are heterotrophs, can respond to the wavelengths of light from UV-C to far-red; however, until recently, only one photoreceptor class of the blue light sensors had been identified in the fungi. Similarly, Blumenstein et al. (2005) has reported a red light sensing via a phytochrome in the model fungus *Aspergillus nidulans*, where it has been suggested that a phytochrome type of system may be operative in these fungi. The phytochromes are photoreceptors that sense the red and far-red light through photo-interconversion between the two stable conformations. This distinct feature is mediated by the chromophores. The phytochromes were thought to be confined to the photosynthetic organisms including the cyanobacteria, but have been recently discovered in the heterotrophic bacteria and in fungi, where little is known about their functions.

Conclusions

The studies on effect of the light revealed that the incubation in total darkness was most effective in inducing the pigment production. The colonies grown under the direct illumination resulted in no pigment production; thus, postulating the existence of photoreceptors responsive to dark and light in this fungus. Physiological and morphological response of the fungi towards different wavelength of light suggested that a phytochrome type of system may be operative in this organism. In this experiment we did not study the effect of light intensity. It could be possible that the intensity of light used in the present study caused inhibition but lower intensities could be favorable for pigment production. Therefore, many things remain to be done for comprehensive understanding of the light induced regulation of pigment biosynthesis, but we expect that the present study will provide a potent insight toward this goal. The study also promises the possibility of identification of light-regulated genes and the unraveling of possible functional interplays between the different light control systems.

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