JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Nov. 2013. Vol. 7(Spl. Edn.), p. 671-678

Effect of Blue Light on the Growth, Culture Morphology, and Pigment Production of *Monascus*

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(Received: 27 September 2013; accepted: 04 November 2013)

Blue light is an environmental signal that can regulate most organisms, including the physiological processes of fungi. In this paper, the effects of blue light on the culture morphology, spore formation, and red pigment production of *Monascus* were investigated. Blue light restricted the formation of aerial hyphae, affected the colony morphology, delayed and increased the formation of conidia, reduced asci, and initiated the sexual cycle at the early stages of growth. It also reduced red pigment production in aerial hyphae and spores. However, 12 d of blue light exposure did not affect red pigment production in culture medium and total pigment production. These findings suggest that *Monascus* can sense blue light through photoreceptors and respond by modifying colony morphology, changing spore formation, and decreasing red pigment production in aerial hyphae.

Key words: Blue light, *Monascus*, Culture morphology, Spore formation, Light perception.

With the growing consumer perception that natural colorants are safe, manufacturers have moved toward natural and non-synthetic food colorants[1]. Thus, safe, naturally occurring edible coloring agents, including the pigments extracted from the fungus *Monascus purpureus*, have received considerable attention[2,3]. *Monascus* species, which are used in the production of traditional oriental foods, such as red mold rice, can produce various useful secondary metabolites, including red pigment (natural coloring agent), γ-aminobutyric acid (GABA, an anti-hypertensive drug), and monacolin K (MONK, a cholesterol-lowering drug)[4].

In nature, microorganisms are exposed to a constantly changing physical and chemical environment. Environmental factors, both physical and nutritional, influence many stages of fungal development[5-7]. Light is one of the most important environmental factors. Among living organisms, fungi occupy an important position in the study of light and its effects on development. For several decades, the different responses of fungi to light have been studied extensively in the long-established model system *Neurospora crassa*. In *Neurospora*, light affects various physiological processes, including entrainment and resetting of circadian clock, biosynthesis of photo-protective pigments, induction of asexual conidiospores, development of reproductive structures, and direction of ascospore dispersal[8-11]. In particular, blue-light perception, signal transduction, and related responses in fungi have been widely investigated. Aside from *Neurospora*, some important secondary metabolites are regulated by blue light. Blue light inhibits mycotoxin production in plant pathogenic fungi, such as *Aspergillus flavus*, *Aspergillus parasiticus*, and *Alternaria alternate*[12], and increases ethanol concentration

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in *Saccharomyces cerevisiae*[13]. Given the importance of *Monascus* to the food and pharmaceutical industries, more studies have focused on the effect of light on *Monascus* growth, spore formation, and secondary metabolism (red pigment, GABA, MONK, and citrinin)[14-16].

Traditionally, a photo-bioreactor illuminated by fluorescent light is used to investigate the influence of light intensity on cell growth and metabolite formation. However, the broad spectrum of wavelengths in fluorescent light may obscure the mechanisms of light occurring in microorganisms. Light-emitting diodes (LEDs) could replace fluorescent light as a high-quality light source, with better features that include smaller mass and volume, longer life, and a single wavelength[17, 18]. Thus, an LED photobioreactor equipped with blue LEDs could be used to investigate the effects of blue light on microorganisms.

Miyake et al.[14] reported on the effect of light on *Monascus*. However, little is known about the effect of blue light on *Monascus*. Hence, this study aims to elucidate the influence of blue light on developmental morphology, air hyphal growth, spore formation, and pigment production in aerial and vegetative mycelia in *Monascus* cultured on wort malt extract agar plates.

MATERIALS AND METHODS

Strain and culture

A culture of *Monascus ruber* N (a high pigment-producing strain) was maintained on wort malt extract agar slant, cultured at 30 °C for 7 d to 9 d, preserved at 4 °C, and then sub-cultured once every 3 weeks.

Inoculum preparation

Sterile distilled water (5 ml) was added to a fresh and fully sporulated agar slope culture. The spores were scrapped under strict aseptic conditions. The obtained spore suspension was subsequently inoculated in 50 ml seed medium (3% rice flour, 0.25% KH_2PO_4, 0.2% $NaNO_3$, and 0.1% $MgSO_4 \cdot 7H_2O$) in 250 ml baffled flask. The initial pH of the medium was adjusted to 4.5 with lactic acid. Subsequently, the flask was incubated at 30 °C at 180 rotation min^{-1} for 30 h. The culture was filtered through sterile glass wool, and the spore suspension containing unclumped spores without hyphae was adjusted to 2×10^7 spores per ml. The resulting spore suspension was used as the inoculum.

Observation of *Monascus* growth and culture morphology

To study the effects of blue light on cell development, wort malt extract agar plate cultures inoculated with the same number of spores (5 µl inoculum) were either incubated in total darkness or under direct blue light exposure for 12 d at 30 °C. Colony morphology was directly observed and photographed.

Observation of mycelium and spores

In order to observe the effects of light on spore formation, the mycelial colonies in plate cultures incubated in the dark or under blue light exposure for 6 d. After 6 d of culture, spores including asci and conidia were harvested with 10 mL sterile solution (0.9% NaCl, 0.2% Tween 80) and counted using a counting chamber. The formation and morphology of spores, including asci and conidia, were further examined thoroughly under a scanning electron microscope (SEM, JEOL JSM 5600LV). SEM-digitized photographs with 1000× or 5000× magnification were obtained using an accelerator voltage of 10 kV.

Determination of red pigments in aerial hyphae

Distilled water (3 ml) was added to the mycelial colonies in plate cultures incubated in the dark or under blue light exposure for 12 d. Subsequently, the aerial hyphae and spores were scrapped, and the suspension was added into a flask containing 7 ml absolute alcohol. The solvent and sample were maintained at 30 °C for 24 h and then filtered through membrane filtration (pore size, 0.45 µm). The concentration of red pigments in aerial hyphae was estimated and expressed as optical density units (U) at 505 nm multiplied by its dilution factor[19], with the values converted thereafter to color units.

Determination of red pigments in culture medium plates

Distilled water (30 ml) was added to the plate in which colony aerial hyphae and spores were scrapped. Agar culture medium and substrate mycelium were divided into smaller fragments and boiled. Absolute alcohol (70 ml) was then added after the solution cooled to 30 °C. The solvent and sample were kept at 30 °C for 24 h, kept in a rotary shaker at 200 rpm for 1 h, and then allowed to stand

for 15 min with membrane filtration (pore size, 0.45 µm). The optical density of red pigments obtained from each culture medium plate was measured using the method employed in aerial hyphal pigments measurement.

Calculation of total pigments

The total pigments of a colony in the plate cultures incubated in the dark or under blue light exposure for 12 d represents the red pigment in aerial hyphae plus the pigment in the culture medium plate.

RESULTS AND DISCUSSION

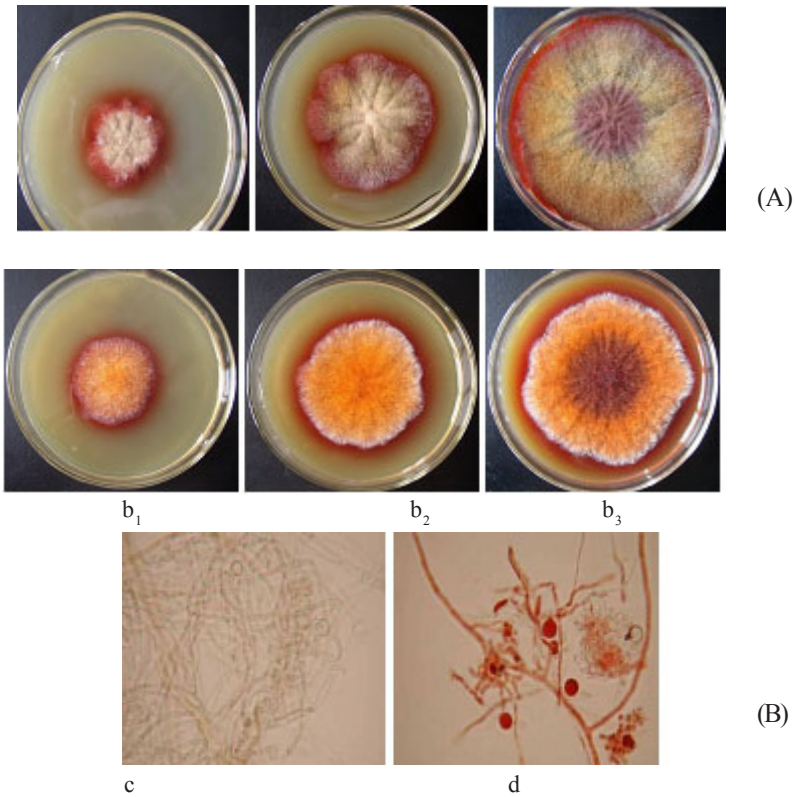
Effect of blue light on *Monascus* culture morphology

Colony formation on wort malt extract agar plates incubated at 30 °C in the dark or under blue light exposure was observed kinetically. The effect of blue light on the cultures was assessed by recording the visible phenotype and by photographing during the 12 d incubation period (Fig. 1-A).

Significant difference in colony color and morphology was found between the colonies cultured in the dark and under blue light exposure. The colonies grown in total darkness exhibited a profuse growth of aerial hyphae, which led to fluffy colonies and highly pigmented mycelia (Figs. 1-A-b_1 to 1-A-b_3). Conversely, the colonies grown

Table 1. Effect of blue light on spore formation

Culture coditions	Number of spores	
	Conidia	Asci
Blue light	114600 ± 680	2300±160
Darkness	78200±410	5400±205



(A) Effect of blue light on *Monascus* culture morphology. (a_1–a_3) Colonies cultured under blue light for 4, 6, and 9 d, respectively. (b_1–b_3) Colonies cultured in total darkness for 4, 6, and 9 d, respectively.

(B) Effect of blue light on *Monascus* mycelium and spores. (c) Mycelium and conidia of *Monascus* exposed to blue light. (d) Mycelium and conidia of *Monascus* cultured in total darkness.

Fig. 1. Effect of blue light on *Monascus* culture morphology

under blue light exposure showed sparse and branched aerial hyphae, with very loose growth than those grown in the dark (Fig. 1-A-a_1 and 1-A-a_2). Although the repression of aerial hyphal development was apparent in the mycelia grown under blue light exposure, the repression was not complete, as evidenced by sparse aerial hyphal development. Moreover, the aerial hyphae in the colonies grown under direct blue light exposure initially appeared pale and then turned scarlet towards the center of the colony (Figs. 1-A-a_1 to 1-A-a_3). By contrast, the aerial hyphae of the colonies grown in total darkness were initially yellow, turned orange, and then finally turned dark red (Figs. 1-A-b_1 to 1-A-b_3). Aerial hyphae, sporophores, and spores grew perpendicular into the air from the vegetative hyphae. Thus, they were irradiated directly by blue light, with a distinct pattern of blue light effect. The mycelium at the tips of the colonies cultured in the dark and under blue light exposure for 4 d at 30 °C was then observed under a light microscope (Fig. 1-B). Microscopic examination revealed that the spores and both aerial and reproductive hyphae turned red in the dark (Fig. 1-B-d) but became pale under blue light exposure (Fig. 1-B-c). This finding may be attributed to the fact that incubation in total darkness is effective in inducing pigments production and that no pigments production occurs under direct blue illumination, this result was in agreement with Miyake *et al*. (2005) and Babitha *et al*. (2008). However, if blue light absolutely inhibited the formation of *Monascus* pigments, the substrate surrounding the colony at the beginning and the aerial hyphae of the later culture under blue light exposure would not be red (Fig. 1-A). We inferred that the pigments in aerial hyphae and spores exposed to blue light was degraded or secreted into the extracellular region. While the stability of monascus red pigments under blue light and darkness had no apparently difference (data not shown). In order to prove that the pigments in aerial hyphae exposed to blue light was more easily secreted into the extracellular region, part plates cultured under darkness for 7 d were transferred to blue light illumination, the other part remain cultured under darkness. The plates were further incubated under blue light exposure for 4 h, the dark red aerial hyphae previously cultured in darkness turned pale. The pigments in aerial hyphae and in plate culture medium cultured under blue light exposure and in the darkness after being transferred from darkness to blue light for another 4 h, was determined (Fig. 2).

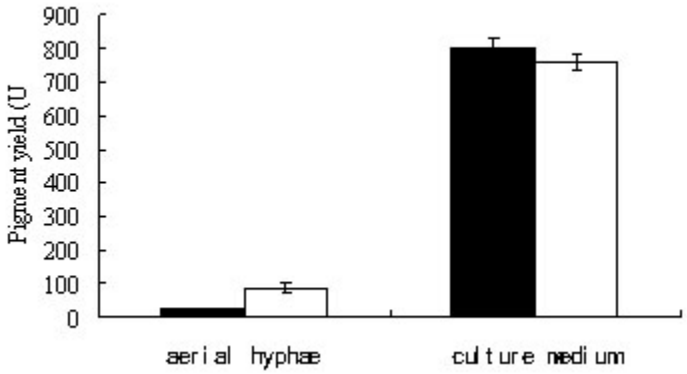


Fig. 2. The pigments in aerial hyphae and culture medium after being transferred from darkness to blue light for another 4 h. (black bars) The pigments in aerial hyphae and culture medium cultured under blue light after being transferred, (white bars) The pigments in aerial hyphae and culture medium remain incubated in darkness after being transferred

After being transferred for another 4 h of culture, the pigments yield in aerial hyphae incubated in darkness (85.7 U) was apparently higher than that under blue light exposure (20.6 U). However, the productivity (760.5 U) in culture medium was lower than that under blue light exposure (800.9 U). These findings illustrated that the pigments in aerial hyphae exposed to blue light was more easily secreted into the extracellular region. However, in the later culture, the aerial

hyphae in the colonies grown under direct blue light exposure turned scarlet towards the center of the colony (Fig. 1-A-a_3), this could be explained by that *Monascus* adapted to blue light, causing the accumulation of red pigment in aerial hyphae, this result was in agreement with the findings of Lee *et al*. (2006). These observations indicated that blue light not only affected the formation of pigments in aerial hyphae but also the culture morphology of *Monascus*. In addition, *Monascus* was found to be capable of sensing blue light through photoreceptors that are responsive to blue light. Following perception, an internal signal was generated, which was subsequently transported via a signal transduction chain. As a result, the pigments production in aerial hyphae was secreted, and the culture morphology of *Monascus* was affected. This finding is in agreement with the observed effects of blue light on *Monascus* pigments yield.

Effect of blue light on spore formation

Monascus, a homothallic fungus, can produce spores both asexually and sexually. Under normal conditions, *Monascus* forms oval or pyriform aleiuroconidia, single or in a chain up to three to four. Conidiospores can survive sudden, often drastic, and stressful changes in their environment[20]. Miyake *et al*.[14] indicated that blue light could stimulate the germination of conidia. Lee *et al*.[21] howed that blue light represses the asexual development and spore release in *Magnaporthe oryzae*. The formation of spores, including asci and conidia at the tips of the colonies cultured in darkness and under blue light exposure for 6 d at 30 °C was then counted (Table 1).

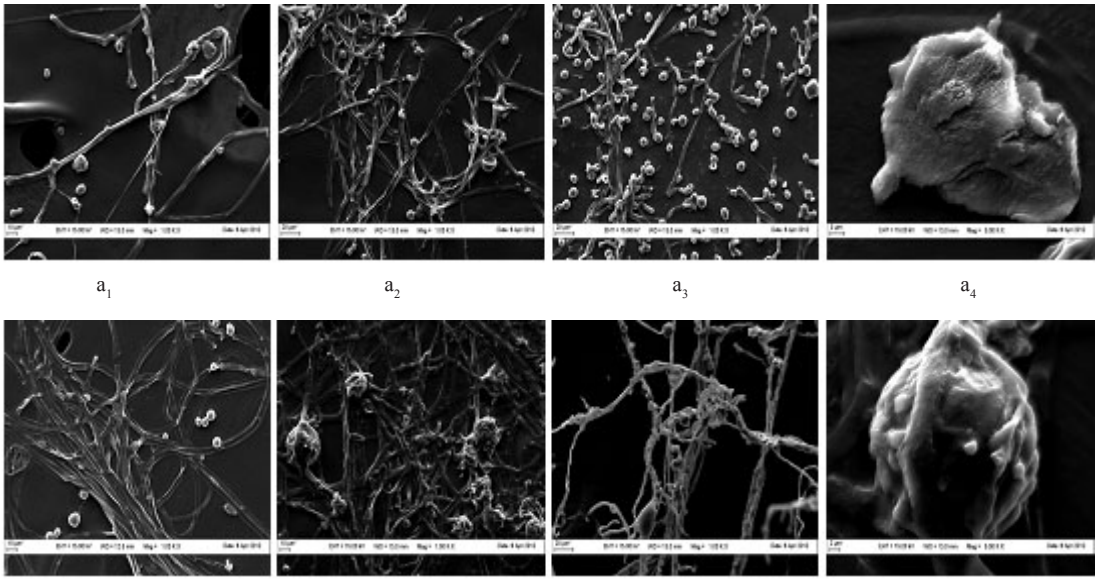
The number of conidia under blue light (114600 ± 680) was more than that in the dark (78200±410), whereas the number of asci under blue light (2300±160) was less than that in the dark (5400±205). These observations indicate that *Monascus* is able to sense blue light, and respond with reducing asci and increasing conidia. Further confirmation of the effect of blue light on the formation and morphology of conidia and asci of *Monascus* was obtained through SEM (Fig. 3). According to the position of the aerial hyphae, the colonies cultured in darkness and under blue light exposure for 6 d were divided into edge, near edge, and central parts. The hyphae observed from the edge to the center were increasingly older. Under normal conditions, the reproduction of *Monascus* cells followed the completion of asexual cycle and the initiation of sexual cycle. Through SEM, conidias and ascis were found at the edge aerial hyphae with the colony cultured under blue light, whereas only conidia were observed in the colony cultured in darkness. Many ascis appeared at the near-edge aerial hyphae of the colony cultured in darkness, whereas none was observed in the colony exposed to blue light. Moreover, many conidia appeared at the central aerial hyphae of the colony cultured under blue light, whereas none was found in the colony cultured in darkness. In addition, the morphologies of the asci that formed under blue light and in darkness were different. The ascospore wall of the asci in the plate incubated under blue light exposure was thicker than that incubated in darkness. These observations indicated that *Monascus* sexual cycle was initiated at the early stage of growth. The formation of conidia in the colony exposed to blue light was delayed. Many conidia were found at the center of the colony cultured under blue light exposure for 6 d. The presence of conidia was reported to be related to pigment formation. The center of the colony appeared red, affirming the effect of blue light on *Monascus* culture morphology.

Effect of blue light on growth and pigments yield

Studies on the effect of blue light on the growth and pigments yield of *Monascus* are necessary for controlling the cellular metabolism and optimization of certain biosynthetic products. The diameter of the colony cultured in total darkness or under blue light exposure was employed to evaluate the effect of blue light on *Monascus* growth. The diameter of the colonies cultured under different conditions was measured every 24 h from the third day (Fig. 4-d).

After 3 d of culture, the diameter of the colony cultured under blue light and in the dark was almost the same. After 4 d to 11 d of culture, the diameter of the colony cultured under blue light grew larger than that cultured in the dark. After 12 d of culture, the diameter of the colony cultured under blue light and in the dark was almost the same again.

The red pigments in aerial hyphae and in plate culture medium, as well as the total red

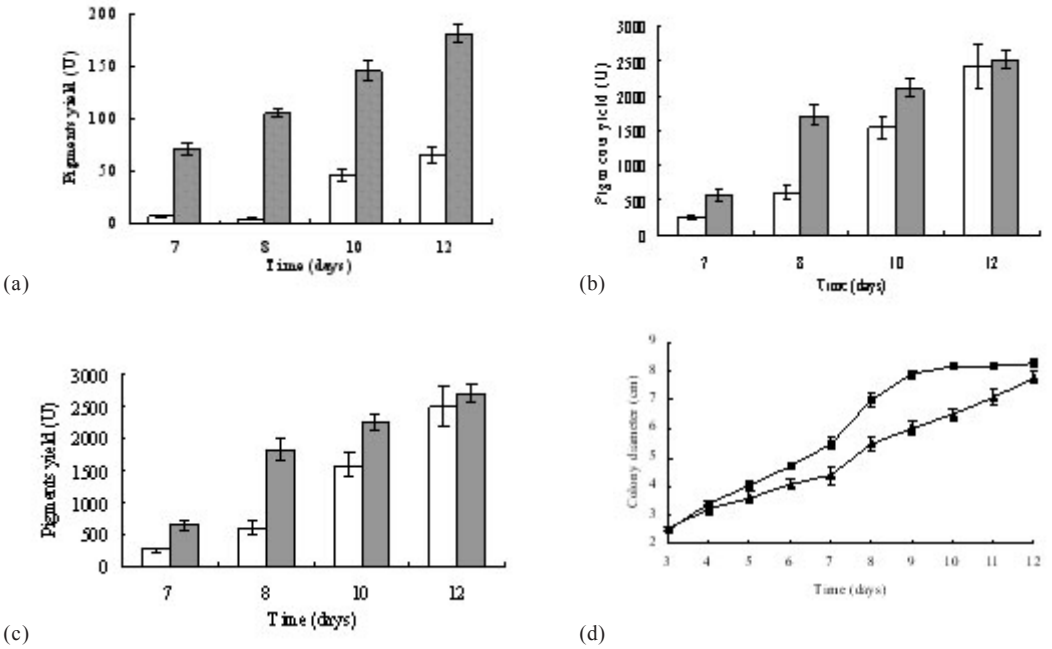


a_1 a_2 a_3 a_4

b_1 b_2 b_3 b_4

(a_1–a_3) SEM image of aerial hyphae at the edge, near the edge, and at the center of the colony cultured under blue light. (b_1–b_3) SEM images of aerial hyphae at the edge, near the edge, and at the center of the colony cultured under darkness. (a_4 and b_4) SEM images of asci under blue light and in the dark.

Fig. 3 Scanning electron microscope (SEM) image of the effect of blue light on the formation and morphology of conidia and asci of *Monascus*



(a–c) Red pigment production in aerial hyphae, culture medium and total red pigment in the plate cultured under blue light or in darkness, (black bars) cultured in darkness, (white bars) exposed to blue light.(d) Colony diameter cultured under blue light and dark, (■) diameter of the colonies cultured under blue light, (▲)diameter of the colonies cultured in total darkness

Fig. 4. Effect of blue light on growth and pigment yield

pigments in the plate cultured under blue light exposure and in the darkness, was determined (Figs. 4-a to Figs. 4-c). The yields of red pigments in aerial hyphae, in the culture medium and the total red pigments in the plate cultured for 7 and 8 d under blue light exposure were apparently lower than those in total darkness. After 12 d of culture, red pigments production in aerial hyphae incubated in total darkness (181.44±8.1U) was approximately threefold of that under blue light exposure (65.19±7.2 U). Meanwhile, the productivities in culture medium and total red pigments production in the plate cultured under blue light exposure and in darkness for 12 d were almost the same. These observations indicated that blue light affected red pigments yield in aerial hyphae throughout the entire period of culture, but not the red pigments yield in culture medium and total pigments by the end of culture. If blue light had an absolute inhibitory effect on red pigments synthesis in *Monascus*, the production of red pigments in the culture medium and the total production in the plate would be very low compared with their production in darkness. Nevertheless, these results showed otherwise. Blue light inhibited the production of red pigments in aerial hyphae because red pigments has a maximum absorbance at 490 nm to 500 nm, which is close to the wavelength of light produced by blue LED (470 nm). To protect *Monascus* from blue light damage, this response was observed. Whether the observed effect of blue light on red pigments production is a biological process requires further study.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Natural Science Foundation of China (No. 31330059 and 31171729).

REFERENCES

1. Carvalho, J.C., Pandey, A., Babitha, S., Soccol, C.R. Production of *Monascus* biopigments: an overview. *Agro. Food Ind. Hi. Tec.*, 2003; **14**(6): 37–42.
2. Lee, Y.K., Chen, D.C., Chauvatcharin, S., Seki, T., Yoshida, T. Production of *Monascus* pigments by a solid-liquid state culture method. *J. Ferment. Bioeng.,* 1995; **79**(5): 516–518.
3. Babitha, S., Soccol, C.R., Pandey, A. Solid-state fermentation for the production of *Monascus* pigments from jackfruit seed. *Bioresour. Technol.,* 2007; **98**(8): 1554–1560.
4. Jozlová, P., Martínková, L., KYen, V. Secondary metabolites of the fungus *Monascus*: a review. *J. Ind. Microbiol. Biotechnol.,* 1996; **16**(3): 163–170.
5. Kües, U., Liu, Y. Fruiting body production in *Basidiomycetes*. *Appl. Microbiol. Biotechnol.,* 2000; **54**(2): 141–152.
6. Romani, L., Bistoni, F., Puccetti, P. Adaptation of *Candida albicans* to the host environment: the role of morphogenesis in virulence and survival in mammalian hosts. *Curr. Opin. Microbiol*., 2003; **6**(4): 338–343.
7. Schneper, L., Duvel, K., Broach, J.R. Sense and sensibility: nutritional response and signal integration in yeast. *Curr. Opin. Microbiol.,* 2004; **7**(6): 624–630.
8. Bahn, Y.S., Xue, C.Y., Idnurm, A., Rutherford, J.C., Heitman, J., Cardenas, M.E. Sensing the environment: lessons from fungi. *Nat. Rev. Microbiol*., 2007; **5**(1): 57–69.
9. Corrochano, L.M. Fungal photoreceptors: sensory molecules for fungal development and behaviour. Photochem. *Photobiol. Sci*., 2007; **6**(7): 725-736.
10. Herrera-Estrella, A., Horwitz, B.A. Looking through the eyes of fungi: molecular genetics of photoreception. *Mol. Microbiol.,* 2007; **64**(1): 5–15.
11. Idnurm, A., Heitman, J. Light controls growth and development via a conserved pathway in the fungal kingdom. *PLoS, Biol,* 2005; **3**(4): e95.
12. Haggblom, P., Unestam, T. Blue light inhibits mycotoxin production and increases total lipids and pigmentation in *Alternaria alternate*. *Appl. Environ. Microbiol*., 1979; **38**: 1074–1077.
13. Shu, C.H., Huang, C.K., Tsai, C.C. Effects of light wavelength and intensity on the production of ethanol by *Saccharomyces cerevisiae* in batch cultures. *J. Chem. Technol. Biotechnol.,* 2009; **84**(8): 1156–1162.
14. Miyake, T., Mori, A., Kii, T., Okuno, T., Usui, Y., Sato, F., Sammoto, H., Watanabe, A., Kariyama, M. Light effects on cell development and secondary metabolism in *Monascus*. *J. Ind. Microbiol. Biotechnol*., 2005; **32**(3): 103-108.
15. Babitha, S., Carvahlo, J.C., Soccol, C.R., Pandey, A. Effect of light on growth, pigment production and culture morphology of Monascus purpureus in solid-state fermentation. *World J. Microbiol. Biotechnol.,* 2008; **24**(11): 2671–2675.
16. Velmurugan, P., Lee, Y.H., Venil, C.K., Lakshmanaperumalsamy, P., Chae, J.C., Oh, B.T. Effect of light on growth, intracellular and

extracellular pigment production by five pigment-producing filamentous fungi in synthetic medium. *J. Biosci. Bioeng*., 2010; **109**(4): 346–350.

17. Bula, R.J., Morrow, R.C., Tibbitts, T.W., Barta, D.J., Ignatius, R.W., Martin, T.S. Light-emitting diodes as a radiation source for plants. *Hort Science*, 1991; **26**(2): 203–205.
18. Brown, C.S., Schuerger, A.C., Sager, J.C. Growth and photomorphogenesis of pepper plants under red light-emitting diodes with supplementalblueor far-red lighting. *J. Am. Soc. Hort. Sci.,* 1995; **120**(5): 808–813.
19. Johns, M.R., Stuart, D.M. Productions of pigments by *Monascus* in solid culture. *J. Ind. Microbiol*., 1991; **8**(1): 23–28.
20. Shinohara, M.L., Correa, A., Bell-Pedersen, D., Dunlap, J.C., Loros, J.J. *Neurospora* Clock-Controlled Gene 9 (*ccg-9*) Encodes Trehalose Synthase: Circadian Regulation of Stress Responses and Development. *Eukaryot Cell,* 2002; **1**(1): 33–43.
21. Lee, K., Singh, P., Chung, W.C. Light regulation of asexual development in the rice blast fungus, *Magnaporthe oryzae*. *Fungal Genet. Biol*., 2006; **43**(10): 694–706.