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Effect of colour LEDs on mycelia growth of *Aspergillus ficuum* and phytase production in photo-fermentations

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ABSTRACT

Aspergillus ficuum grown on plates and in liquid cultures were illuminated by a white fluorescent light and four different colour LED lights (white, blue, green and red) to evaluate the regulation of LED lights on fungal growth. Biomass conversion, pellet size and phytase activity were examined. In liquid culture, luminous intensity was highly correlated with the rate of biomass conversion but did not affect pellet size. The white fluorescent light contained several different wavelengths, and therefore, its effect on *A. ficuum* represents the cooperative effect of these wavelengths. Strong luminance of a white fluorescent light inhibited growth of *A. ficuum* mycelia on plates, whereas white LED light enhanced growth. The development of mycelia was also inhibited by blue LED light and enhanced by red LED light illumination. Investigating the effect of LED lights on the growth of *A. ficuum* could provide evidence on the luminous intensity that is sufficient for regulating fermentation by light.

1. Introduction

Light energy is essential to life on earth. It is a crucial environmental signal that regulates the development and physiology of all organisms, including fungi. Light regulates the growth, metabolism and reproduction of fungi and thus is important for the survival and dissemination of fungi [1]. Therefore, the effects of light on fungal development have been intensively investigated for decades, particularly in model fungi such as the Basidiomycetes genus *Coprinus* and the Zygomycetes genus *Phycomyces* [2,3].

At the molecular level, the function of white collar genes wc-1 and wc-2 of the ascomycetes species *Neurosporo crassa* in sensing light has been well characterised by Linden et al. [4,5]. Blue light regulates the circadian rhythm, pigment production and sexual fruiting body formation of *N. crassa* by regulating the expression of wc-1 or wc-2, which encode proteins with several conserved domains, such as the PAS and LOV domains. The wc-1 protein functions as a blue light receptor through a specialised PAS domain and works with wc-2 to act as a transcription factor [1,4,6]. A small protein, VIVID, interacts with blue light through a LOV domain, which causes *N. crassa* to be sensitive to light [7–9]. Similar light sensing system associated with white collar proteins in *Aspergillus nidulans* was recently discovered by Purschwitz et al. and reviewed by Bayram et al. [10,11].

Macroscopically, image analysis can be a powerful technology to examine the effect of light on fungal growth. It allows the assessment and measurement of structure from examination of electronic images. In particular, it has been used to measure the size and shape of filamentous microorganisms [12]. Many industrial fermentations involving submerged fungal cultures have been extensively examined, with the intention of relating gross morphology and productivity to processing. The types of morphologies identified range from freely dispersed hyphae to pellets. It is well established that illumination can alter the morphology of some fungi, especially the length of spores and density of mycelia [13,14]. Early studies have revealed that fungal spores tend to be longer when exposed to light than in the dark [14]. Studies on the light-dependent development of reproductive structures in *A. nidulans* have revealed that sexual development can be promoted by darkness, whereas asexual sporulation can be stimulated by illumination. The induction of conidiation of *A. nidulans* can also be regulated by various wavelengths of lights, such as red and blue light [9,15].

Various secondary metabolites that are important in fungal fermentation are also known to be regulated by blue light. For example, mycotoxin production is inhibited by blue light in *Aspergillus flavus*, *Alternaria alternate* and Moanscus spp. [16,17]. Phytic acid is the major means of phosphorus storage in many seeds and cereals. Up to 80% of phosphorus in plants has been reported to be in the form of phytate phosphorus. *In vivo*, phytic acid prevents protein degradation by forming protein complexes and can bind to multivalent cations, such as Zn^{2+}, Ca^{2+} and Fe^{2+}, and decrease their

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bio-availability [18,19]. Phytase hydrolyses phytic acid in cereals and thus increases the bio-availability of these cations [20,21].

Microbial phytase has been used to reduce phytic acid levels in some cereals, such as canola meal, that do not contain the enzyme. *Aspergillus ficuum* is able to produce phytase and reduce phytic acid content in canola oil. However, the production of phytase by *A. ficuum* can be influenced by the amount of phosphate in the substrate [22–24]. We previously established a strategy to improve the production of phytase in liquid cultured *A. ficuum* [C.K. Chen, Ph.D. thesis, National Central University, Tauyuan, 2009]. Qualitative relationships between wavelength and illuminance on phytase production in *A. ficuum* were defined. The morphology of the pellets was examined, and the diameters were calculated and used as an index parameter for the performance of fermentation under light. The production of phytase by *A. ficuum* is positively correlated with pellet diameter. Pellet morphology can be examined by image analysis, which can be used as a quick, convenient method for the quality control of secondary metabolite fermentation under light control [12,25].

While the effect of light on fungi has been previously investigated, the light sources used in these studies were not well defined due to equipment limitations. The objects were illuminated by light from a fluorescent or halogen lamp passing through a colour filter. The bandwidths of the visible light spectra were too broad to specifically define the light source. The qualities of light sources in the experiments were questioned because of the complexity and variability of the light, and the results of these studies should be considered preliminary [26].

The aim of this work is to demonstrate the effect of light on the growth of solid cultured *A. ficuum* to examine whether mycelia growth on plates correlate with the production of phytase. The quality of the light sources was carefully defined in this study to avoid any deviation in light source. The effect of light on the growth of fungi on solid media culture may also act as an index for mycelia fermentation [27]. Understanding the effect of light on mycelia growth on plates may provide important information in the working cultures, which are the liquid cultures for homogeneous growth of the fungus, and solid culture of photo-fermentations. Examining the density and shapes of mycelia on plates would save time and reduce costs of media selection, working culture and solid culture.

2. Materials and methods

2.1. Fungus and culture medium

A. ficuum (Reichert) Hennings (BCRC 32870; NRRL3135) strain was purchased from the Bioresource Collection and Research Centre (BCRC; Sinchu, Taiwan) and used throughout this study. The fungus was cultured and preserved on slants of potato dextrose agar (PDA, Difico Co.) at 28 °C and retransmitted every 10 days. Prior to the liquid culture experiments, the fungus was adapted to the liquid culture by inoculating *A. ficuum* from the plates into the liquid culture medium in an Erlenmeyer flask at 28 °C. The liquid culture medium used in this study was a modified yeast peptone dextrose medium (YPD, Difico Co.) with 10 g of sucrose, 2 g of yeast extract, 3 g of diammonium sulphate ($(NH_4)_2SO_4$), 2 g of peptone, 0.5 g of potassium chloride (KCl), 0.01 g of magnesium sulphate heptahydrate ($MgSO_4{\cdot}7H_2O$) and 0.01 g of ferrous sulphate heptahydrate ($FeSO_4{\cdot}7H_2O$) per litre.

All reagents used in this study were reagent and analytical grade except when specified. Cultures were adapted to liquid media several days prior to cultivation experiments by inoculating 2 ml of *A. ficuum* (5% (v/v)) into 40 ml of liquid culture in an aluminium-shielded 250-ml Erlenmeyer flask (pre-sterilized at 120 °C for 20 min); the cultures were incubated at 28 °C (LTI-1000SD, EYELA, Japan) [28].

2.2. Submerged liquid cultivation of A. ficuum for photo-fermentation

Each of 5 ml of *A. ficuum* cultures were inoculated into 100 ml of YMD in sterilized 250-ml Erlenmeyer shake flasks at an inoculation ratio of 5% (v/v). The flasks were shaken at 150 rpm and incubated at 28 °C for 120 h. The medium was supplemented with 0.1% carbopol-940 and 10 g/l sucrose.

A fluorescent lamp (10 W, China Electric Manufacture Co., Taiwan) installed inside an orbital shaking incubator (Kansin Instruments Co., Taiwan) was used as the light source. The illuminance of the fluorescent lamp was regulated by a voltage regulator and measured by an illuminance metre, which could be adjusted from 0 to 2500 lux inside the reactor. Samples of culture fluid taken at specified times were assayed for the measurement of biomass, sucrose consumption, and phytase activity. The mycelial pellets of *A. ficuum* acquired from the cultured media were placed on a slide and examined under a microscope (Leica DME, Germany) at a 10 × 10 power. For each examination, 10 pellets were imaged by a digital camera (Nikon Coolpix 4500, Japan), and the diameters were calculated following the method described by Cox et al. [12]. The tests were performed in triplicate, and the results represent the average of the three measurements.

2.3. Solid-state cultivation with colour LED illumination

Similar to the liquid cultures, a schematic of the solid-state cultivation is shown in Fig. 1. Instead of the fluorescent lamp, the light sources used for the solid-state cultivation were light-emitting diode lamps (LED). The LED lamps (Vitalux Co., Taiwan) were suspended on top of the plates. Four different colour LED lights were used: blue (λ_{max} = 461.5 nm, $W_{1/2}$ = 22.0 nm), green (λ_{max} = 529.0 nm, $W_{1/2}$ = 32.0 nm), red (λ_{max} = 631.5 nm, $W_{1/2}$ = 14.5 nm) and white (broad and mixing spectrum). The 1000 lux of light irradiance was measured and arranged in front of the LEDs. An identical experiment was performed in the dark as a control experiment. The experimental units were covered by a light-proof black cloth to avoid the leakage of ambient light.

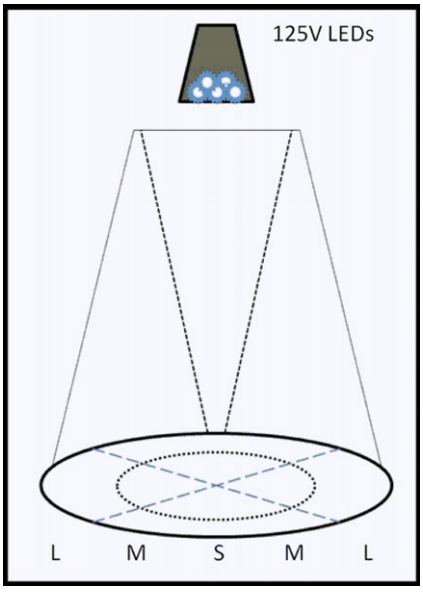

Fig. 1. Setup of the fungal solid-state (agar plate) cultivation under illumination by LEDs in an isolated box.