

EFFECTS OF LIGHT WAVELENGTHS AND COHERENCE ON BASIDIOSPORES GERMINATION

Natalia Poyedinok^{* 1}, Anatoly Negriyko², Oksana Mikhailova³

Address(es):

¹ Institute of Food Biotechnology and Genomics of National Academy of Sciences of Ukraine, Ukraine, Osipovskogo 2a, 04123, Kyiv, Ukraine, tel +38 044 462 72 59.

² Institute of Physics of National Academy of Sciences of Ukraine, Ukraine, Nauki prosp. 46, 03028, Kyiv, Ukraine.

³ M.G. Kholodny Institute of Botany of National Academy of Sciences of Ukraine, Ukraine, Tereshchenkivska St.2, 01601 Kyiv, Ukraine.

*Corresponding author: poyedinok@ukr.net

doi: 10.15414/jmbfs.2015.4.4.352-357

ARTICLE INFO

Received 20. 8. 2014
Revised 9. 12. 2014
Accepted 15. 12. 2014
Published 1. 2. 2015

Regular article



ABSTRACT

The effects of light wavelengths and coherence on basidiospore germination of *Agaricus bisporus*, *Flammulina velutipes*, *Ganoderma applanatum*, *Ganoderma lucidum*, *Hericium erinaceus*, *Lentinus edodes* and *Pleurotus ostreatus* have been studied. Short-term low-intensity irradiation by coherent (laser) light wavelength 488.0 nm and 632.8 nm at doses 45 and 230 mJ/cm² has significantly increased the number of germinated basidiospores. It has established that there are differences in the photosensitivity not only between species but also between strains. Spores irradiation by 514.5 nm light has been either neutral or inhibitory. A comparative analysis of basidiospores sensitivity to laser and LED light has also been conducted. To stimulate germination of basidiospores and growth of monokaryons the most suitable solution was to use red coherent and incoherent light of 632.8 nm and 660,0 nm for *A. bisporus*, *G. applanatum* and *P. ostreatus*, red and blue coherent light of 632.8 nm and 488,0 nm for *F. velutipes*, and both red and blue laser and LED light *G. lucidum* and *H. erinaceus* and for *L. edodes*. No essential difference of a continuous wave mode and intermittent mode light effect at the same doses and wavelength on spore germination were revealed. Light influence has reduced germination time and formation of aerial mycelium on agar medium as compared to the original value and increased the growth rate of monosporous isolates. Characterization of basidiospores photosensitivity and development of environmentally friendly stimulating methods of their germination is important for creating highly effective technologies of mushrooms selection and cultivation.

Keywords: Basidiospores, photosensitivity, mushrooms, monospores, germination, spectra, artificial light

INTRODUCTION

One of the major trends in the modern mycology and biotechnology is reproduction of various macromycetes in the culture, preservation of them in collections and selection of highly productive strains with desired characteristics for further application. This is why many researchers pay attention to obtaining pure cultures from basidiospores (Fries, 1987; D'Enfert, 1997; Bulesco et al., 2005; Kalmis and Kalyoncu, 2006; Dulay et al., 2012) and have difficulties in their laboratory germination (Kreisel and Shcauer, 1987; Feofilova et al., 2004). Therefore, the issue of activation of their germination remains relevant today.

In addition, in recent years, spores of macromycetes have attracted considerable attention in terms of their use for medicines with useful and often with unique properties. The bioactivity of spores may be even higher than that of a fruit body (Min et al., 1998; Zhu et al., 2000; Xin et al., 2002; Pei-Yu et al., 2012).

In accordance with their biological functions, basidiospores have certain morphological, physiological, biochemical and genetic characteristics: the structure of cell walls, cell nuclei heterothallism, spare nutrients, dehydration, the minimum level of metabolic, and dormancy. To overcome this state a cell must navigate to the stage of active cell metabolism. This means that activation of oxidative processes, aerobic respiration, protein synthesis, and finally formation and growth of a new morphological structure should happen. These stages of the spore germination process of some fungi have been well illustrated (D'Enfert, 1997).

Various ways to activate germination of basidiospores of higher basidiomycetes during dormancy by the heat treatment, adding in the medium the activated carbon and / or yeast, amino acids, organic acids, mono- and disaccharides, alcohols, flavonoids, hydrogen peroxide have been described (Kikuchi et al., Vidyapin et al., 2007; Dulay et al., 2012; Karadeniz et al., 2013). However, these methods are generally time consuming and do not always lead to the desired result.

For the majority of macromycetes, despite the fact that they are not phototrophic organisms, light is an important morphogenetic factor. For decades, at least about

100 species of fungi from different taxonomic groups that are responsive to the light have been studied (Deploey, 1995; Fries, 1987; Moor, 2002; Moor et al., 2008, 2011; Laringuet and Dunand, 2005; Corrochano and Galland, 2006; Purschwitz et al., 2006; Herrera-Estrella and Horwitz, 2007; Kritskiy et al., 2010; Tisch and Schmoll, 2010; Corrochano, 2011). Significant factual material so far accumulated proves that most of the photophysiological phenomena in fungi are associated with the regulation of their growth and individual development. The fungi reaction to light during ontogenesis is particularly diverse at the transition of organism to the next new phase of individual development, e.g. the spores germination and formation of sporogenous structures. The nature of light signal response depends on duration, intensity, wavelength and other spectral characteristics of light, features of an organism and may be either stimulating or suppressing. The regulating effects of light with various wavelengths and intensities on growth, reproduction, ripening and secondary metabolism of fungi on different stages of morphogenesis were widely studied (Tisch and Schmoll, 2010; Corrochano, 2011; Moor, 2002; Moor et al., 2008, 2011; Poyedinok, 2013). The photobiology of fungi has been extensively investigated, but in recent years the identification of the first fungal photoreceptor, WC-1 in the ascomycete *Neurospora crassa*, and the discovery that similar photoreceptors are required for photoreception in other ascomycete, basidiomycete and zygomycete fungi has allowed the molecular characterization of light reception and the early steps of signal transduction in a number of model fungi. Blue light in the type of light was most closely associated with fungal photomorphogenesis. In addition, blue light can activate fungal metabolic pathways for the direct growth of fungal structures (Idnurm et al. 2010). Several types of photoreceptors have been described in fungi (Herrera-Estrella and Horwitz, 2007; Kamada T. et al., 2010; Corrochano, 2011; Yang T. and Dong C., 2014). The completion of several fungal genomes allowed the identification of additional fungal photoreceptor genes, many of which were unexpected, such as red light absorbing photoreceptors, phytochromes, additional blue-light absorbing photoreceptors, cryptochromes, and rhodopsins. There is enough information about the sensitivity of the mushrooms to light of various wavelengths and intensities in the range from 350 to 730 nm on the vegetative

mycelium growth and the formation of reproductive structures as well as the lack of similar investigations of the mushrooms basidiospores photosensitivity.

The low-intensity laser irradiation is used for stimulation of various biological processes in experimental biology, biotechnology and medicine. It has been proved that photosensitivity of different cells (prokaryotes, primitive and higher eukaryotes) to low-intensity monochromatic light has a universal character, which implies the existence of the very same molecular mechanism with the primary photoacceptors (Karu, 2008). Experimental data testify that a relatively small dose (10^2 - 10^3 mJ/m²) and short-time exposure cause a long-lasting macroeffect (Pastore et al., 1996; Vacca et al., 1996; Gagliardi et al., 1997; Greco et al., 1999, Karu, 2011). One of the most controversial, topical and widely discussed issues is the question of whether the coherence of laser radiation has additional benefits from a biostimulation, as compared with monochromatic or quasimonochromatic light from a conventional light source (i.e. properly filtered light from a lamp) or LED (light-emitting diode) with the same wavelength and intensity as a laser beam (Hode, 2005; Karu, 2008, 2011; Zalevsky and Belkin, 2011). Nowadays, the problem of importance of light coherence in biological experiments is not as hot a topic as it used to be 10-20 years ago. There is no data on the study of specificity of laser light effect on the fungi and the comparative analysis their sensitivity to light of different nature.

Therefore, it is of great practical and scientific interest to provide the comparative study the effect of light with different wavelengths and coherence on macromycetes basidiospores photosensitivity and the subsequent growth of monospores.

MATERIAL AND METHODS

Fungal Strains

Pure strains of *Agaricus bisporus* (JE Lange) Imbach (strains 5418.5422, 5437), *Flammulina velutipes* (Curtis) Singer (strains 1668.1974, 2038), *Ganoderma applanatum* (Pers.) Pat. (strains 920, 1672, 1899), *Ganoderma lucidum* (Curtis) P. Karst. (strains 921, 1670, 1887), *Hericiium erinaceus* (Bull.) Pers. (strains 963, 968, 1756), *Lentinus edodes* (Berk.) Singer (strains 520, 712, 1992), *Pleurotus ostreatus* (Jacq.) P. Kumm. (strains 527, 531, 553) were kindly supplied by the Mushrooms Collection of the M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine (IBK) (Buchalo et al, 2011).

Substrates and Obtaining of Fruit Bodies

Fruit bodies were obtained on the following substrates: *A. bisporus* – mushrooms synthetic compost (calculating per 1 Ton of dry straw), consisting of 900 Kg of dry chicken manure, 100 Kg of ammonium nitrate, 1 Ton of dry wheat straw; *F. velutipes*, *G. applanatum*, *G. lucidum* – aspen sawdust (60%) and wheat bran (40%); *H. erinaceus* beech sawdust (60%) and corn grits (40%); *L. edodes* beech sawdust (60%) and corn grits (40%); *P. ostreatus* wheat straw 40%, alder sawdust 40%, wheat bran 20%

Collection and Germination of Basidiospores

Basidiospores were collected from healthy basidiocarps. The mature fruit body previously was wiped with 70% alcohol. The spore prints of *A. bisporus*, *F. velutipes*, *L. edodes* and *P. ostreatus* were obtained by laying down the open basidiocarp in sterile Petri dishes. Basidiospores of *G. applanatum*, *G. lucidum* and *H. erinaceus* were obtained by hanging fruit bodies in sterile containers over sterile foil. The plates and containers were incubated at ambient room temperature to allow detachment of basidiospores from the basidium. After that, the foil with spores was transferred into sterile Petri dishes. After spores had been collected, 5 ml sterile water was added to the Petri dishes under aseptic conditions. Petri dishes containing 5 ml of sterile water were gently shaken to collect the maximum number of spores. Water was then transferred into sterile test tubes as a basidiospore suspension. For preparation of a spore suspension, 1 ml aliquots from the basidiospore suspension were transferred into sterile test tubes containing 9 ml sterile water (1:10). Spore numbers per 1 ml of each dilution were counted with a haemocytometer (Neubauer improved "Optic Labor") in three replicates. The desired concentrations of spores were obtained by serial dilutions. Spores were applied to the medium immediately after receiving the suspension. One milliliter of spore suspension was uniformly distributed over the water agar surface (*A. bisporus* and *H. erinaceus* 968, 1756 - 1000 spores per 1 Petri dish, due to the poor initial spores germination, and *F. velutipes*, *G. applanatum*, *G. lucidum*, *L. edodes*, *P. ostreatus* and *H. erinaceus* 963 - 100 spores per 1 Petri dish,) using sterile pipettes under aseptic conditions. As *A. bisporus* spores do not germinate on water agar, a process of their germination was studied on Goodwin medium (Feofilova et al., 2004). In order to prevent a bacterial infection, the following antibiotics were added to the medium: 200 units of penicillin and 100 units of streptomycin in 1 ml of medium. Ten replicates were prepared and all of them were incubated in the dark at 26 +1 °C. The incubation time was dependent on the species of mushroom.

Irradiation

Irradiation of spores was performed directly in Petri dishes. Spores without irradiation were used as control.

The gas lasers were used as the source of coherent visible light. The helium-neon laser LGN- 215 type with a wavelength of 632.8 nm, production "Polaron" Co., Ukraine and argon ion laser (modified model LGN- 106M1 manufactured by NPO "Plasma", Russia) with wavelengths of 514,5 nm and 488,0 nm were used. The laser beam has been expanded to the Petri dishes area or, in the case of spreading of spores at more local area, the expanded beam size has been matched with size of spore spreading. The laser power density was measured by Digital Optical Power and Energy Meter PM 100D, Thorlabs Inc. with Standard Photodiode Power Sensor S120C, Si, 400-1100 nm. The exposure dose was defined by product of power density and time of irradiation. Due to the rather wide spread of power density provided by used light sources (from 0,15 mW/cm² for He-Ne laser up to 5 mW/cm² for Ar⁺ laser), the exposure time has been selected in accordance with the dose.

As noncoherent light sources the light emitted diodes with central wavelength 430 nm and 660 nm and linewidth 25 nm respectively were applied. The AlGaInN LEDs YSH-FRGG-IA produced by China Young Sun Led Technology Ltd were used. Each LED microchip comprises three-diode light source with a separate independent power control in each spectral range (430 nm, 522 nm and 660 nm.). The light with wavelength 430 (blue) and 660 (red) nm was used in experiments. The maximal electrical power of each LED diode was 1 W. Irradiation doses were selected as 45, 230 and 650 mJ/cm². Its value was defined according to our previous experiments (Poyedinok et al., 2000, 2008) and by analysis of other authors' results (Sommer et al. 1989; Moor, 2002; Chen et al. 2009; Tisch and Schmoll, 2010). The above light wavelengths were selected because photoreceptors systems of fungi adapted to visible light in the range from 350 to 730 nm (Moor, 2002; Moor et al., 2008, 2011; Tisch and Schmoll, 2010; Corrochano, 2011). This spectral range is rather well represented by selected wavelengths light (blue, green, red regions).

The helium-neon laser and LED lamps have been used both in a continuous wave (cw) mode and intermittent mode (meander with a duration of 1 ms and a repetition period of 2 ms).

In all variants of the experiments, the conditions of equal light exposures on mushroom spores were chosen. For all kinds of light sources, the energy density on the sample surface was the same. We have selected these common conditions for all experiments due to the fact that under conditions where the mechanism of action of low-intensity radiation on spores has not been set yet, we have aimed to determine the quality differences of equal doses impact on the studied biological objects of radiation with different spectral composition, various temporal characteristics and varying degrees of coherence. The time of spores treatment by light is determined by light source power and target irradiation dose. For the usually used 90 mm Petri dish, the net dose of light irradiation is approximately 18-20 J and typical treatment time of single sample varies from 1 to 20 minutes.

Measurement of Mycelia Growth

Germinated spores (10 in every variant) lying separately were transferred into new Petri dishes containing wort agar to measure mycelial growth rate. All Petri dishes were incubated at 26 +1 °C, accurately recording the time of the growth beginning (the appearance of aerial mycelium). The radii of the colonies were measured in two mutually perpendicular directions every day until complete fouling surface of the nutrient medium in Petri dishes. The average rate of growth was determined on the basis of 8-12 parallel measurements.

Statistical Analysis

The results were presented as median (inter-quartile range). The differences between groups were analyzed using the non-parametric Wilcoxon signed-rank test, and a "p" value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Our results show that a short-term low-intensity irradiation by coherent (laser) light wavelength 488.0 nm and 632.8 nm at doses 45 and 230 mJ/cm² had a stimulating effect and significantly increased the number of germinated spores in the studied macromycetes species. It has been revealed that the optimal irradiation dose is 230 mJ/cm². The results have established significant differences in the photosensitivity not only between species but also between strains. This suggests that the sensitivity to light have strain-specific signs. We can assume that individual reaction of strains to light impacts, due to their different geographical origin and epigenetic features, have been formed under the influence of the environment.

Irradiation by 514,5 nm light in the same doses were either neutral (*P.ostreatus*, *G. aplanatum*, *A. bisporus*) or inhibitory (*H. erinaceus*, *L. edodes*, *G. Lucidum*, *F. velutipes*) (Tab 1).

Table 1 Spores photosensitivity of different species and strains of macromycetes

Strain	Spore germination, % of total									Control without irradiation
	632,8 nm			514,0 nm			488,0 nm			
	45 mJ/cm ²	230 mJ/cm ²	650 mJ/cm ²	45 mJ/cm ²	230 mJ/cm ²	650 mJ/cm ²	45 mJ/cm ²	230 mJ/cm ²	650 mJ/cm ²	
<i>Agaricus bisporus</i>										
5422	7,9±0,4	15,9±0,7	0	0,8±0,2	0,7±0,1	0	1,2±0,3	4,9±1,3	0	0,8±0,1
5418	6,8±1,1	10,8±1,8	0	2,3±0,3	2,3±0,3	0	3,6±0,5	7,5±0,3	0	3,3±0,5
5437	4,9±0,6	13,9±2,2	0	1,0±0,2	1,0±0,2	0	2,0±0,4	6,1±0,8	0	1,2±0,2
<i>Flammulina velutipes</i>										
1668	66,6±2,0	78,9±5,4	2,7±0,1	5,6±1,1	3,6±1,1	0	28,8±1,4	64,6±4,3	0	7,2±1,3
2038	54,5±1,9	84,3±3,6	0,9±0,1	2,4±0,4	1,2±0,2	0	36,5±0,8	82,7±5,4	0	5,4±0,9
1974	60,0±3,3	70,8±4,0	0	2,9±0,1	2,0±0,1	0	44,2±0,7	72,8±3,3	0	3,9±0,5
<i>Ganoderma aplanatum</i>										
920	43,6±5,7	77,4±3,5	1,0 ±0,3	9,0±2,6	8,5±1,4	0	8,7±0,8	10,5±1,8	0,1±0,1	8,8±1,2
1672	31,4±3,9	54,0 ±3,0	0	7,0±4,2	7,9±0,8	0	6,5±0,3	7,6±0,5	0,7±0,2	6,6±0,6
1899	50,3±2,7	55,5 ±1,8	0	3,4±0,4	3,6±0,2	0	2,3±0,4	3,3±0,1	0	2,9±0,7
<i>Ganoderma lucidum</i>										
921	60,7±3,7	72,4±3,7	9,5±0,6	10,6±2,1	9,8±1,4	0,6±0,2	48,9±1,4	58,3±5,5	5,9±1,8	15,6±2,0
1670	57,7±4,8	69,9±1,5	4,0±0,3	1,1±0,4	4,2±0,4	1,1±0,1	52,2±2,4	66,6±2,6	1,8±0,7	5,1±0,9
1887	75,2±1,9	81,7±4,1	0	0,6±0,2	0,6±0,1	0,3±0,1	60,5±3,7	77,3±5,9	0,3±0,1	2,6±0,3
<i>Hericium erinaceus</i>										
968	2,75±0,3	11,42±1,1	0	<0,1	<0,1	0	1,86±0,1	5,13±0,3	0	<0,1
1756	<0,1	0,8 10 ⁻²	0	<0,1	<0,1	0	<0,1	<0,1	0	<0,1
963	82,0±4,3	98,1±0,9	0	12,9±0,4	9,2±0,5	0	76,3±3,3	90,5±2,5	0	13,6±0,6
<i>Lentinus edodes</i>										
520	45,5 ±1,0	90,3 ±5,8	0	24,8 ±4,7	10,6 ±2,9	0	67,4±5,7	76,8 ±3.1	6.8±0,5	26,6 ±2.1
712	77,9 ±2,1	85,2 ±2,1	2,5 ±0,7	38,3 ±2,1	19.6±2,5	2,7±0,7	80,3±2,4	88.9±2,8	12,6±1,6	59.9±3,5
1992	80,7 ±3,4	97,3 ±4,4	0,9 ±0,2	55,5 ±4,7	24,7±3,9	0,3±0,1	88,3±1,7	96,7±6,9	5,0±0,3	64,7±0,9
<i>Pleurotus ostreatus</i>										
527	16,8±1,1	92,4±3,6	0,9±0,2	12,9±1,9	14,5±1,6	0	18,3±1,9	35,6±3,8	0	13,3±0,9
531	17,6±0,9	93,9±4,0	0,3±0,1	10,8±0,9	11,7±2,3	0	19,7±1,7	26,9±4,5	0	9,8±0,7
553	22,7±2,8	90,2±2,9	0	15,0±2,3	17,1±3,3	0	20,5±2,3	31,2±4,9	0	16,5±2,3

This is not consistent with the data obtained by McCracken (1982). He reported on the significant inhibition of *Pleurotus sapidus* spore germination by day and green light, but blue, yellow and red considered ineffective. This difference can be explained by the specificity of the impact of the low-dose coherent light on the biological objects used in our studies. Since there is still no unequivocal opinion about the specificity of the laser light effect on biological objects, we conducted a comparative study of the impact of

coherent (laser) and incoherent (LEDs) light on basidiospore germination and growth of monosporous isolates. Studies of *F. velutipes*, *G. lucidum*, *H. erinaceus* spore germination showed that they are more sensitive to the coherent light than to incoherent one (Tab 2). Spores of *A. bisporus* and *G. applanatum* were practically insensitive to noncoherent light with central wavelength 430 nm.

Table 2 Coherence effect on the basidiospores germination

Mushroom	Spore germination, % of total				Control
	Incoherent light		Coherent light		
	660,0 nm	430,0 nm	632,8 nm	488,0 nm	
<i>A. bisporus</i> , 5422	10,6±1,3	1,2±0,1	15,9±0,7	4,9±1,3	0,8±0,1
<i>F. velutipes</i> , 1974	22,7±1,6	25,5±2,3	70,8±4,0	72,8±3,3	3,9±0,5
<i>G. applanatum</i> , 1899	36,6±2,0	2,4±0,3	55,5 ±1,8	3,3±0,1	2,9±0,7
<i>G. lucidum</i> , 1887	60,7±2,7	65,8±3,2	81,7±4,1	77,3±5,9	2,6±0,3
<i>H. erinaceus</i> , 963	53,6±3,9	56,6±2,4	98,1±0,9	90,5±2,5	13,6±0,6
<i>L. edodes</i> , 520	88,7±4,8	81,9±5,3	90,3 ±5,8	76,8 ±3.1	26,6 ±2.1
<i>P. ostreatus</i> , 531	92,7±3,8	13,6±2,2	93,9±4,0	26,9±4,5	9,8±0,7

Such beneficial effects of laser light on the germination of *F. velutipes*, *G. lucidum*, *H. erinaceus* spores could be connected with its deeper penetration into their cellular structure. The possible impact of light coherence in photo stimulation of biological processes has been discussed a lot (Karu, 2003; 2011; Rubinov and Afanas'ev, 2005). The experiments on different biological objects

have demonstrated the substantiated higher biological activity of coherent laser radiation, possibly due to the mechanism of the spatial inhomogeneity of the laser field. In fact, possible effect of light coherence is likely to be related to the spatial inhomogeneity of scattered coherent light intensity, i.e. the speckle pattern. For the coherent light the partial light fields formed by scattering in the depth of

biological media are still coherent, but are distributed by the random phase difference. These fields are interfering and producing the interference pattern with strong local nonhomogeneity of light intensity: at the spatial scale, it equals approximately to laser wavelength and the light intensity varies from zero to the level exceeding the mean. The high gradients of light field and nonhomogeneous deposition of laser light energy can lead to the modification of photobiological processes, local increase of temperature, light pressure effects on the separate cells or cells fragments like “optical tweezer” mechanism etc. (Karu, 2003, 2008; 2011; Hode, 2005; Zalevsky and Belkin, 2011).

However, spores of studied strains *L. edodes* were equally responsive to coherent and incoherent light. *P. ostreatus* equally responded to coherent and incoherent light in the red part of the spectrum, but was almost twice as sensitive to the coherent light in the blue part. Further, understanding the reasons for such photoresponses requires further studies.

The possible impact of coherent interaction of light with biological molecules can be expected in the experiments with short light pulses. Today it has been experimentally shown that femtosecond laser pulses can be used to control the photochemical processes in biological molecules (Prokhopenko et al., 2006; Brumer and Shapiro 2012). Dependence of the biological effect of the duration of the light pulse is shown experimentally on living cells.

We have conducted a comparative study of basidiospores photosensitivity to light in continuous and intermittent modes (helium-neon laser – 632,8 nm and light emitting diodes – 660,0 nm). Only insignificant differences were observed in action of continuous wave and intermittent light on spore germination at the same dose and wavelength (Fig 1 A and B). Currently there is no agreement on whether continuous wave or pulsed light gives a better effect and on what main factors the pulse parameters is to be chosen (Hashmi et al., 2010). Therefore, this issue deserves further systematic studies.

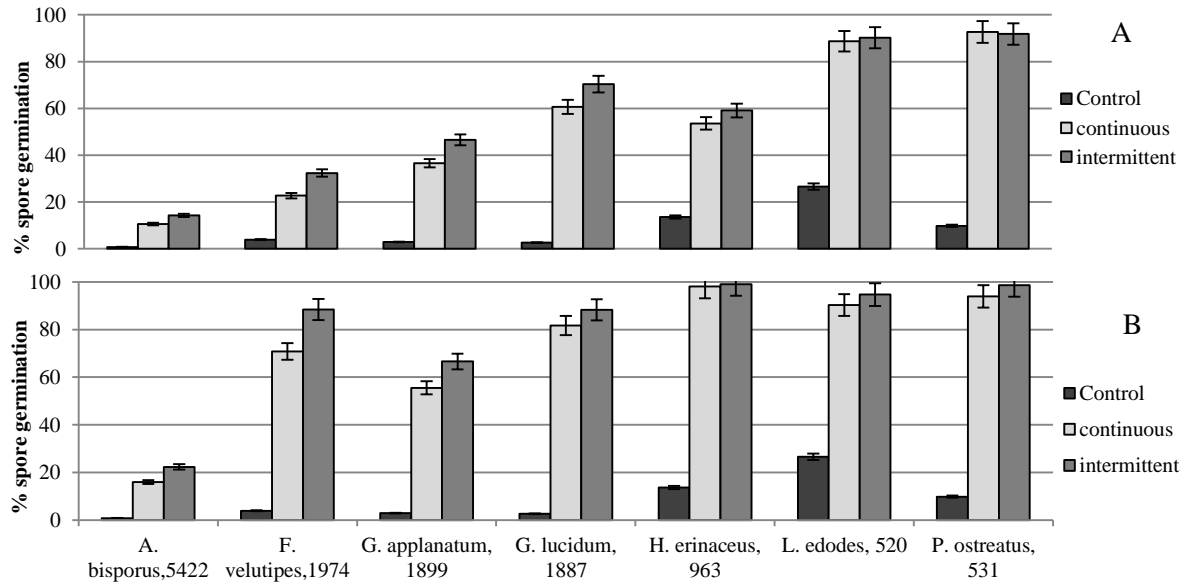


Figure 1 Light nature effect on spores germination; A – incoherent light, B – coherent light

It can be noted that the stimulatory effect of irradiation was usually more effective for a lower initial rate of spore germination. Similar dependence was observed by other researchers in stimulating the growth of microorganisms by laser irradiation (Karu, 2003). Seasonality of growth stimulating effects was identified, i.e. the stimulation may take place only when the cell proliferation activity (the culture growth rate) is not maximized. In summer, when the cultural

growth was accelerated, the photostimulation effect practically was not observed. It was the highest in winter and medium in spring and autumn. For mushrooms whose spores germinate for a long time, we found a reduced germination time and formation of aerial mycelium on agar medium after light influence as compared to the original value (Tab 3).

Table 3 Terms of basidiospores germination and aerial mycelium formation

Mushroom	Basidiospores germination, %, day		Aerial mycelium formation, day	
	Control	Irradiation (632.8 nm) 230 mJ/cm ²)	Control	Irradiation (632.8 nm) 230 mJ/cm ²)
<i>A. bisporus</i> , 5422	6 (0,8%)	4 (15,9%)	9	7
<i>H. erinaceus</i> , 963	9 (<0,1%)	5 (11,4%)	10	7
<i>L. edodes</i> , 520	8 (26,6%)	6 (90,3%)	11	8

In addition, irradiation by artificial light has activated the growth of monosporous isolates (Fig 2). It is of great importance in breeding. As we have already mentioned, at this stage of our researches we also have found the confirmation of

specificity and greater efficiency of coherent light (632.8 nm and 488 nm) as compared to non-coherent (660.0 nm and 430.0 nm).

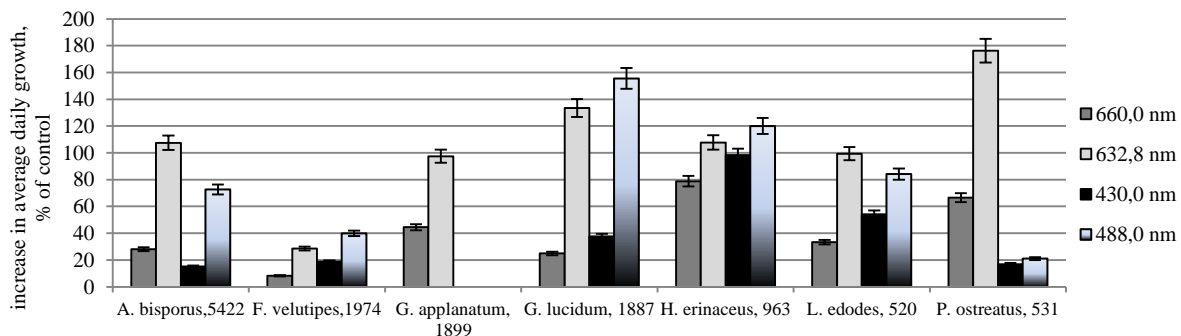


Figure 2 Effect of irradiation on the growth rate of monosporous isolates

CONCLUSION

Effects of light wavelengths and coherence on basidiospore germination of *A. bisporus*, *F. velutipes*, *G. applanatum*, *G. lucidum*, *H. erinaceus*, *L. edodes* and *P. ostreatus* have been investigated in this study for the first time. To stimulate germination of basidiospores and growth of monokaryons the most suitable solution is to use red coherent and incoherent light of 632.8 nm and 660,0 nm for *A. bisporus*, *G. applanatum* and *P. ostreatus*, red and blue coherent light of 632.8 nm and 488,0 nm for *F. velutipes*, *G. lucidum* and *H. erinaceus* and both red and blue laser and LED light for *L. edodes*. The comparative study the effect of light with different wavelengths and coherence on macromycetes basidiospores activity is one of the important stages of understanding the widely discussed issues concerning additional benefits from a biostimulation of the coherence of laser radiation, as compared to monochromatic light with the same wavelength and intensity as a laser light. Such studies should be continued. Intraspecific sensitivity basidiospores of different strains varied considerably and this should be taken into account when developing methods for photostimulation of other practically valuable macromycetes. Although only insignificant differences have been observed in action of continuous wave and intermittent light on spore germination, further research in this direction can be both of scientific and practical interest. It has been established that light influence has reduced basidiospores germination time and formation of aerial mycelium as compared to the original value and increased the growth rate of monosporous isolates. Analysis of the obtained data gives serious grounds for further research aimed at studying the duration of preservation of the photo induced basidiospores activity and its transfer to other stages of morphogenesis. Characterization of basidiospores photosensitivity and development of environmentally friendly stimulating methods of their germination is important for creating highly effective technologies of mushrooms selection and cultivation.

REFERENCES

- BRUMER, P., SHAPIRO, M. 2012. Molecular response in one-photon absorption via natural thermal light vs. pulsed laser excitation. *Proceedings of the National Academy of Sciences of the United States of America*, 10(48), 19575–19578. <http://dx.doi.org/10.1073/pnas.1211209109>
- BUHALO, A.S., MYTROPOLSKAYA, N.YU., MIKHAILOVA, O.B. 2011. Culture collection of mushrooms IBK. Kyiv: Altpress, 100 p. ISBN-978-966-542-487-1. [In Ukrainian]
- BULSECO M.G., ABELLA E., REYES R.G. 2005. Morphogenesis of *Schizophyllum commune*, a wild edible mushroom of Mt. Nagpale, Abucay, Bataan, Philippines. *Journal of Nature Studies*, 4(1), 20–28.
- CHEN, C.H., RINGELBERG, C.S., GROSS, R.H., DUNLAP, J.C., LOROS, J.J. 2009 Genome-wide analysis of light-inducible responses reveals hierarchical light signalling in *Neurospora*. *EMBO J.* 28(8), 1029–1042. <http://dx.doi.org/10.1038/emboj.2009.54>
- CORROCHANO, L.M., GALLAND, P. 2006. Photomorphogenesis and gravitropism in fungi. In *The Mycota*, vol. I: Growth, Differentiation and Sexuality. Berlin; Springer-Verlag, pp.233-259. ISBN 3-540-57781-5.
- CORROCHANO, L.M. 2011. Fungal photobiology: a synopsis. *IMA Fungus*. 2(1), 25-28. <http://dx.doi.org/10.5598/imafungus.2011.02.01.04>
- D'ENFERT, C. 1997. Fungal spore germination: Insights from the molecular genetics of *Aspergillus nidulans* and *Neurospora crassa*. *Fungal Genetics and Biology*, 21(2), 163–172. <http://dx.doi.org/10.1104/pp.47.5.729>
- DEPLOEY, J.J. 1995. Some factors affecting the germination of *Thermoascus aurantiacus* ascospores. *Mycologia*, 87(3), 362-365. <http://dx.doi.org/10.2307/3760833>
- DULAY, R.M., CABRERA, E.C., KALAW, S.P., REYES, R.G. 2012. Optimal growth conditions for basidiospore germination and morphogenesis of Philippine wild strain of *Lentinus tigrinus*(Bull.) Fr. *Mycosphere*, 3(6). <http://dx.doi.org/10.5943/mycosphere/3/6/6> (http://www.mycosphere.org/pdfs/MC3_6_No6.pdf)
- FEOFILOVA, E.P., TERESHINA, V.M., GARIBOVA, L.V., ZAVYALOVA, L.A., MEMORSKY, A.S., MARYSHOVA, N.S. 2004. Basidiospores of Agaricus bisporus germination. *Applied Biochemistry and Microbiology*. 40(2), 220-226. [in Russian]
- FRIES, N. 1987. Ecological and evolutionary aspects of spore germination in the higher basidiomycetes. *Transaction of the British Mycological Society*, 88, 1– 7. [http://dx.doi.org/10.1016/S0007-1536\(87\)80179-1](http://dx.doi.org/10.1016/S0007-1536(87)80179-1)
- GAGLIARDI, S., ATLANTE, A., PASSARELLA, S. 1997. A novel property of adenine nucleotides: sensitivity to helium-neon laser in mitochondrial reactions. *Biochemistry and Molecular Biology International*, 413, 449-460.
- GRECO, M., PERLINO, E., PASTORE, D., GUIDA, G., MARRA, E., QUAGLIARIELLO, E. 1991). Helium-neon laser irradiation of rat liver mitochondria gives rise to a new subpopulation of mitochondria: isolation and first biochemical characterization. *Journal of Photochemistry and Photobiology*, 10(1-2), 71-78. [http://dx.doi.org/10.1016/1011-1344\(91\)80213-2](http://dx.doi.org/10.1016/1011-1344(91)80213-2)
- HASHMI, T., HUANG, Y.-Y., SHARMA, S.K., KURUP, D.B., TABOADA, L.D., CARROLL, J.D., HAMBLIN, M.D. 2010. Effect of Pulsing in Low-Level Light Therapy. *Lasers in Surgery and Medicine*, 42(6), 450–466.
- HERRERA-ESTRELLA, A., HORWITZ, B.A. 2007. Looking through the eyes of fungi: molecular genetics of photoreception. *Molecular Microbiology*. 64(1), 5-15. <http://dx.doi.org/10.1111/j.1365-2958.2007.05632.x>
- HODE L. (2005).The importance of the coherence. *Photomedicine and Laser Surgery*. 23(4), 431-434. <http://dx.doi.org/10.1089/pho.2005.23.431>
- KALMIS E., KALYONCU F. 2006. Variations in the isolates obtained from basidiospores of commercial mushroom *Lentinula edodes* (Shiitake). *International Journal of Science & Technology*, 1(2), 99-103.
- KAMADA, T., SANO, H., NAKAZAWA, T., NAKAHORI, K. 2010. Regulation of fruiting body photomorphogenesis in *Coprinopsis cinerea*. *Fungal Genetics and Biology*, 47(11), 917-921. <http://dx.doi.org/10.1016/j.fgb.2010.05.003>
- KARADENIZ, E., SARIGULLU, F.E., UNTAC, I. 2013. Isolation and germination of *Ganoderma lucidum* basidiospores and effect of H₂O₂ on the germination of spores. *Food, Agriculture and Environment*, 11(1), 745-747.
- KARU T.I. 2003. Cellular mechanism of low power laser therapy: new questions. In: *Lasers in Medicine and Dentistry*. Vitgraf: Rieka, 79-100, ISBN 978-1-4200-8290-6.
- KARU, T.I. 2008. Universal cellular mechanism of laser biostimulation: photoactivation of respiratory chain enzyme cytochrome oxidase. *Holography: basic research, innovative projects and nanotechnology* (Proceeding of the work of XXVI school of coherent optics and holography. - Irkutsk: "Papyrus", 2008 - S.156-175. [In Russian].
- KARU, T.I. 2011. Light coherence. Is this Property Important for Photomedicine? <http://www.photobiology.info/Coherence.html>
- KIKUCHI, K., MATSUSHITA, N., SUZUKI, K., HOGETSU, T. 2007. Flavonoids induce germination of basidiospores of the ectomycorrhizal fungus *Suillus bovinus*. *Mycorrhiza*, 17(7), 563-570. <http://dx.doi.org/10.1007/s00572-007-0131-8>
- KREISEL, H. & SHCAUER, F. 1987. Methoden des mykologischen laboratoriums. New York: Cristav Fischer Verlag, 181 p.
- KRITSKIY, M.S., TELEGINA, T.A., VECHTOMOVA, Y.L., KOLESNIKOV, M.P., LYUDNIKOVA, T.A., GOLUB, O.A. (2010). Photoexcited molecules of flavin and pterin coenzymes in evolution. Overview. *Biochemistry*, 75(10), 1348-1366. <http://dx.doi.org/10.1134/S0006297910100020>
- LARINGUET, P., DUNAND, C. 2005. Plant Photoreceptors: Phylogenetic Overview. *Journal of Molecular Evolution*, 61(4), 559-569. <http://dx.doi.org/10.1007/s00239-004-0294-2>
- MCCRACKEN, F. 1982. Some factors affecting basidiospore germination of *Pleurotus sapidus*. *Canadian Journal of Botany*, 60(9), 1658-1661. <http://dx.doi.org/10.1139/b82-215>
- MIN, B.S., NAKAMURA, N., MIYASHIRO H., BAE, K.W., HATTORI, M. 1998. Triterpenes from the spores of *Ganoderma lucidum* and their inhibitory activity against HIV-1 protease. *Chemical and Pharmaceutical Bulletin*, 46(10), 1607–1612. <http://dx.doi.org/10.1248/cpb.46.1607>
- MOOR, D. 2002. Fungal morphogenesis. Cambridge University Press, 469 p. ISBN-10: 0521552958
- MOORE, D., GANGE, A.C., GANGE, E.G., BODDY, L. 2008. Fruit bodies: their production and development in relation to environment. In *Ecology of Saprotrophic Basidiomycetes*. Amsterdam. 386 p. ISBN-10: 0123741858
- MOORE, D., ROBSON, G.D., TRINCI, A.P.J. 2011. 21st Century Guidebook to Fungi. Cambridge, UK: Cambridge University Press, 639 p. ISBN-13: 978-0521186957
- PASTORE, D., DI MARTINO, C., BOSCO, G., PASSARELLA, S. 1996. Stimulation of ATP synthesis via oxidative phosphorylation in wheat mitochondria irradiated with helium-neon laser. *Biochemistry and Molecular Biology International*, 39(1), 149-157.
- PEI-YU, H., JIUN-LIANG, CH., HSING-YU, CH., SIEN-HUNG, Y. 2012. Extract of sporoderm-broken germinating spores of *Ganoderma lucidum* activates human polymorphonuclear Neutrophils via the P38 mitogen-activated protein kinase pathway. *Chang Gung Medical Journal*, 35(2), 140-148.
- POYEDINOK, N.L., POTEKINA, J.V., BUCHALO, A.S., NEGRIYKO, A.M., GRYGANSKY, A.P.H. 2000. Stimulation with low-intensity laser light of basidiospore germination and growth of monocaryotic isolates in Medicinal Mushroom *Heriicum erinaecus* (Bull.: Fr.) Pers.(Aphyllorhizomycetidae). *Internaional Journal of Medicinal Mushrooms*, 2(4), 339-342. <http://dx.doi.org/10.1615/IntJMedMushr.v2.i4.140>
- POYEDINOK, N.L., MYKHAILOVA, O.B., BUCHALO, A.S., NEGRIYKO, A.M. 2008. Light Regulation of Growth and Biosynthetic Activity of Ling Zhi or Reishi Medicinal Mushroom, *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst. (Aphyllorhizomycetidae), in Pure Culture. *Internaional Journal of Medicinal Mushrooms*, 10(4), 369-378. <http://dx.doi.org/10.1615/IntJMedMushr.v10.i4.100>
- POYEDINOK, N.L. 2013. Artificial light using in mushroom cultivation. *Biotechnologia Acta*, 6(6), 58-70. [in Russian]
- PROKHORENKO, V. I., A. NAGY, A. M., WASCHUK, S. A. 2006. Coherent Control of Retinal Isomerization in Bacteriorhodopsin. *Science*, 313(5791), 1257–1261. <http://dx.doi.org/10.1126/science.1130747>

- PURSCHWITZ, J., MULLER, S., KASTNER, CH., FISCHER, R. 2006. Seeing the rainbow: light sensing in fungi. *Current Opinion in Microbiology*, 9(6), 566–571. <http://dx.doi.org/10.1016/j.mib.2006.10.011>
- RUBINOV, A.N., AFANAS'EV, A.A. 2005. Nonresonance mechanisms of biological effects of coherent and incoherent light. *Optics Spectroscopy*, 98(6), 943–948. <http://dx.doi.org/10.1134/1.1953391>
- SOMMER, T., CHAMBERS, J.A., EBERLE, J., LAUTER, F.R., RUSSO, V.E. 1989. Fast light-regulated genes of *Neurospora crassa*. *Nucleic Acids Research*, 17(14), 5713–5723. <http://dx.doi.org/10.1093/nar/17.14.5713>
- TISCH, D., SCHMOLL, M. 2010. Light regulation of metabolic pathways in fungi. *Applied Microbiology and Biotechnology*, 85(5), 1259–1277. <http://dx.doi.org/10.1007/s00253-009-2320-1>
- VACCA, R.F., MARRA, E., PASSARELLA, S., PETRAGALLO, V.A., GRECO, M. 1996. Increase in cytosolic and mitochondrial protein synthesis in rat hepatocytes irradiated in vitro by He-Ne laser. *Journal of Photochemistry and Photobiology*, 34(2-3), 197-202. [http://dx.doi.org/10.1016/1011-1344\(95\)07280-2](http://dx.doi.org/10.1016/1011-1344(95)07280-2)
- VIDYAPIN, V.I., ZHARIKOVA, G.G., NEFELOVA, M.V., ZHARIKOVA, E.N., KOZMINA, A.O., STASEVYCH, V.M. 2007. Method of higher mushroom basidiospores germination. Patent, RU2188537 [in Russian]
- XIN, L., JIAN-PING, Y., XIAO-JUN, CH. 2002. Antitumor activity of the sporoderm-broken germinating spores of *Ganoderma lucidum*. *Cancer Letters*, 182(2), 155–161. [http://dx.doi.org/10.1016/S0304-3835\(02\)00080-0](http://dx.doi.org/10.1016/S0304-3835(02)00080-0)
- YANG, T., DONG, C., 2014. Photo morphogenesis and photo response of the blue-light receptor gene Cmw-1 in different strains of *Cordyceps militaris*. *FEMS Microbiology Letters*. 352(2), 190-197. <http://dx.doi.org/10.1111/1574-6968.12393>
- ZALEVSKY, Z., BELKIN, M. 2011. Coherence and Speckle in Photomedicine and Photobiology. *Photomedicine and Laser Surgery*, 29(10), 655-656. <http://dx.doi.org/10.1089/pho.2010.2939>
- ZHU, H.S., YANG, X.L., WANG, L.B., ZHAO, D.X., CHEN, L. 2000. Effects of extracts from sporoderm-broken spores of *Ganoderma lucidum* on HeLa cells. *Cell Biology and Toxicology*, 16(3), 201–206. <http://dx.doi.org/10.1023/A:1007663006548>