

## INDUCTION OF ANTIMICROBIAL ACTIVITY OF SOME MACROMYCETES BY LOW-INTENSITY LIGHT

N. L. Poyedinok<sup>1</sup>  
O. B. Mykhaylova<sup>2</sup>  
A. M. Negriyko<sup>3</sup>  
I. A. Dudka<sup>2</sup>  
B. F. Vasilyeva<sup>4</sup>  
O. V. Efremenkova<sup>4</sup>

<sup>1</sup>Institute of Food Biotechnology and Genomics  
of the National Academy of Sciences of Ukraine, Kyiv  
<sup>2</sup>Kholodny of Institute Botany of the National Academy  
of Sciences of Ukraine, Kyiv  
<sup>3</sup>Institute of Physics of the National Academy of Sciences of Ukraine,  
Kyiv  
<sup>4</sup>Gause Institute of New Antibiotics, Moscow, Russian Federation

*E-mail: poyedinok@ukr.net*

Received 17.12.2014

The aim of the work was to study the induction of antimicrobial activity of macromycetes by low-intensity light of different wavelengths and coherence. The objects of investigation were the strains of *Flammulina velutipes* 3923, *Pleurotus ostreatus* 531, *Ganoderma lucidum* 1908 and *G. applanatum* 1552 from Mushrooms Collection of the Kholodny Institute of Botany of the National Academy of Sciences of Ukraine, the test-cultures from Cultural Collections of the Gause Institute of New Antibiotics, All-Union Research Institute of Antibiotics and the All-Russian Collection of Industrial microorganisms. Helium-neon laser with a wavelength of 632.8 nm and an argon ion laser with wavelengths of 488.0 nm and 514.5 nm were used as a source of coherent visible light lasers. For obtaining incoherent light LEDs with emission at a wavelength of 490.0, 520.0 and 634.0 nm were used. It was found that short-term exposure of sowing mycelium by low intensity light with the energy density of 230 MJ/cm<sup>2</sup> in the red and blue wavelength ranges reduced the cultivation period before the appearance of antimicrobial activity and induced the increasing of the culture fluid inhibitory activity against different test-cultures from 20 to 238%. Selected modes of antimicrobial activity photostimulation could be used in biotechnology of submerged cultivation of macromycetes for intensification of technological stages and increasing the yield of the final product.

**Key words:** macromycetes, low intensity lights, lasers, antimicrobial activity, light emitting diodes.

Bacteremia is an actual problem of modern medicine. In recent years a great number of human pathogenic bacteria with drug resistance was found [1]. As a result the number of effective antimicrobial drugs decreases and there is an increasing need in new antibiotics [2, 3]. There are many antibiotic producers among pro- and eukaryotes, but fungi, including higher fungi, can be considered as the largest group of producers which was studied for the last decade [4–11]. However, the investigations of antimicrobial activity of macromycetes are limited by the screening and determination of chemical structure of antibiotics.

One of the requirements to antibiotic producers is submerged cultivation that in case of discovery of the promising compound

can be a base for technology development and its production by synthesis. To reach higher productivity of the processes that would result in the receipt of the target product researchers are looking for optimum conditions of cultivation.

The study of factors that affect antimicrobial activity of macromycetes is limited by selection of nutrient media [12, 13]. Despite the great amount of investigations the search of new environmentally friendly regulators of growth and biological activity of fungi is highly relevant. The light is one of such regulators [14–16]. We have previously shown the possibility of the usage of low-intensity coherent laser and incoherent light from different sources to stimulate the growth and biosynthetic activity of some macromycetes [17].

The aim of this work was to study the induction of antimicrobial activity of some macromycetes, which are producers of biologically active compounds, by low-intensity light of different wavelengths and coherence.

### Materials and Methods

The objects of investigations were four strains of four basidiomycete species from Mushrooms Collection of the Kholodny Institute of Botany of the National Academy of Sciences of Ukraine: *Flammulina velutipes* (Curtis) Singer 3923, *Pleurotus ostreatus* (Jacq.) P. Kumm. 531, *Ganoderma lucidum* (Curtis) P. Karst. 1908 and *Ganoderma applanatum* (Pers.) Pat.1552. Twelve test-cultures were also used for investigations: Gram-positive bacteria (*Bacillus subtilis* ATCC 6633, *B. mycoides* 537, *B. pumilus* NCTC 8241, *Leuconostoc mesenteroides* VKPM B-4177, *Micrococcus luteus* NCTC 8340, *Staphylococcus aureus* FDA 209P (MSSA), INA 00761 (MRSA, clinical isolate), Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Comamonas terrigena* VKPM B-7571 (=ATCC 8461), *Pseudomonas aeruginosa* ATCC 27853) and fungi (*Aspergillus niger* INA 00760, *Saccharomyces cerevisiae* RIA 259).

For the test-cultures the modified Gause medium was used (%): glucose — 1, peptone — 0.5, tryptone — 0.3, agar — 2 and sodium chloride — 0.5.

The medium for submerged cultivation was the following (%): glucose — 1.0, soy flour — 2.0, malt extract «Maltax» (Finland) — 2.0.

Macromycetes, test-cultures *L. mesenteroides* VKPM B-4177, *C. terrigena* VKPM B-7571 (=ATCC 8461), *A. niger* INA 00760 and *S. cerevisiae* RIA 259 were incubated at 28 °C, whereas other test-cultures were incubated at 37 °C. The period of cultivation for test-cultures was 17–20 hours.

The submerged cultivation of macromycetes was carried out in Erlenmeyer flasks (volume 500 ml, volume of the medium — 100 ml) on a rotary shaker (200 rpm). The flasks were inoculated with the culture from the agar medium. The inoculum was presented by 5 wort agar disks 10 mm diameter with the mycelium of 7-day culture. The activity in the culture fluid was determined once a week on the 7<sup>th</sup>, 14<sup>th</sup>, and 21<sup>st</sup> day of growth.

Antimicrobial activity of the cultural fluid was determined by agar diffusion method. Test-cultures (10<sup>7</sup> cells/ml) were inoculated into the cooled molten modified Gause medium. After solidification of the agar medium 100 µl

of the culture fluid was added into wells 9 mm diameter.

To concentrate antimicrobial compounds ethyl acetate was added to the culture fluid (volume ratio 1:2). The mixture was vigorously shaken for 10 min and stored at +4 °C for 20 hours. After that ethyl acetate was removed, the mixture evaporated and the obtained pellet solved in 10% aqueous methanol to give a 150-fold concentration. A solution of the resulting concentrate was applied to filter paper discs (6 mm diameter), the disks were air dried and applied on the surface of the medium with test-culture. Given the amount of concentrate coated on the disk in each sample contained approximately 15 times more substances compared to the substances contained in 100 µl of culture fluid introduced into the well.

Antimicrobial activity was determined by the diameters of the zones of growth delay of test-cultures after incubation, the change of the activity was expressed as a percentage relative to control. Seed mycelium grown on the surface of agar medium was subjected to light exposure. Cultures which were not subjected to the light treatment served as control.

The sources of coherent visible light were gas lasers: helium-neon LGN 215 with radiation at a wavelength of 632.8 nm (red) (“Poliaron”, Lviv, Ukraine) and argon ion (modified model LGN-106M1, Scientific Production Association “Plasma”, Russian Federation) with radiation at a wavelength of 514.5 nm and 488.0 nm. Modification of the laser was performed as an installation of laser resonator Littrow prism instead of one of the mirrors. That gave the possibility to configure the instrument to generate radiation at a selected wavelength. Basic modification of the laser LGN-106 M1 was designed to generate radiation simultaneously at several wavelengths, predominantly 488.0 and 514.5 nm. The laser beam was defocused by lens to the area size of the Petri dish. Light emitting diodes (LED) with a wavelength of 490.0 (blue), 520.0 (green) and 634.0 nm (red) were used as the sources of incoherent light.

Laser power density was measured using a digital optical power and energy meter PM-100D (Thorlabs Inc.) with a standard photodiode sensor power S120C, the working range of 400–1100 nm. Energy dose (light energy incident per unit area) was defined as the product of power density and exposure time. Thanks to a sufficiently broad variation of output power of light sources the exposure was selected according to a predetermined dose of 230 mJ/cm<sup>2</sup>

and varied from 1 to 30 minutes, depending upon the experimental scheme.

In all variants of the experiment we used the conditions of equal doses of light energy on the spores and mycelium, so for all types of light sources, the energy density on the surface of the sample was the same. To provide the same energy irradiation conditions of the samples (when established and generally accepted mechanism of action of low-intensity radiation on the mycelium were absent) it seemed reasonable to determine the qualitative differences of fungi exposure to equal doses of radiation of different spectral composition.

The investigations which were carried out on other biological objects showed that their sensitivity to light of low intensity was universal, implying the existence of the same molecular mechanisms [18]. The obtained data proved that short-term (from fractions of seconds to tens of minutes) low-intensity laser coherent radiation in relatively small doses ( $10^2$ – $10^3$  J/m<sup>2</sup>) promoted persistent macro effect. In our experiments the optical radiation energy was 230 mJ/m<sup>2</sup>. This value was chosen in accordance to the results of our previous studies [17] and the analysis of data obtained by other researchers [19]. As it is known, fungi photoreceptor system is adapted to visible light in the wavelength range from 350 to 730 nm [14, 19, 20] and this spectral series proved our selection of wavelengths (blue, green and red region).

Light exposure of 7-day mycelium was performed directly in Petri dishes with a diameter of 90 mm. In this case the dose was 18–20 J and the period of the light exposure per a single sample was from 1 to 20 min.

Experimental results were expressed as mean  $\pm$  standard deviation from the mean of three replicates. The results were processed using Microsoft Excel 2007 and Anova version 0.98. The values with  $P < 0.05$  were considered as statistically significant.

## Results and Discussion

Short-term exposure of seed mycelium by low-intensity light in red and blue wavelength ranges increased antimicrobial activity macromycetes towards almost all the above mentioned test cultures (Table). Green light either did not cause changes in the level of activity or suppress it. We have previously found antimicrobial activity of strain 531 *P. ostreatus* against Gram-positive bacteria *B. mycoides* 537, *B. pumilus* NCTC 8241, *L. mesenteroides* VKPM B-4177, *M. luteus* NCTC 8340, *S. aureus* FDA 209P (MSSA)

and INA 00761 (MRSA, clinical isolate), as well as against Gram-negative bacteria *E. coli* ATCC 25922, *C. terrigena* VKPM B-7571 (=ATCC 8461) and *P. aeruginosa* ATCC 27853 [17]. At this stage the biosynthesis of the antibiotic component in the control probes was fixed after 14 days of incubation (except components active against MSSA and MRSA), that is in accordance with our previous studies. Exposure by laser (coherent) light (632.8 nm and 488.0 nm) as well as by light-emitting diodes (incoherent) in the same wavelength range induced the appearance of antimicrobial activity on the 7th day of cultivation of *P. ostreatus* against *B. mycoides*, *B. pumilus*, *L. mesenteroides* and *C. terrigena*. Increasing of the antimicrobial activity by more than 100% was observed against *B. pumilus*, *L. mesenteroides*, *C. terrigena*, MRSA, MSSA and *E. coli*. The zone of growth inhibition of *B. mycoides* by culture filtrate which was obtained after 14 days of incubation of *P. ostreatus* irradiated with red laser light increased by 3.8 times compared to the control. Irradiation with blue light increased the zone of growth inhibition of this test-culture almost by 3 times. Low-intensity laser radiation induced 10–40% greater stimulatory effect compared with the LED emission. After irradiation of *P. ostreatus* with green light it was observed the decreasing of antimicrobial activity of the culture fluid against *B. mycoides*, MRSA, *M. luteus* and *P. aeruginosa*. However, exposure in this range with either coherent or incoherent light increased the biosynthesis of antimicrobial components which suppressed the growth of *E. coli*. Based on this, it can be assumed that the green light stimulates this strain to synthesize specific compounds which are active against *E. coli*.

After two weeks of cultivation *F. velutipes* strain revealed antimicrobial activity against a broad spectrum of microorganisms: *B. subtilis* ATCC 6633, *S. aureus* FDA 209P, *S. aureus* INA 00761 and *E. coli* ATCC 2592 and after a week the growth inhibition of *A. niger* INA 00760 was observed. Three antibiotic components were revealed at this strain during the one-stage and two-stage cultivation [5]. Irradiation of seed mycelium of this fungus with red and blue coherent light stimulated its synthesis and accordingly increased the activity against all of the above mentioned microorganisms including micromycete *A. niger* for 60–150%. Stimulation of inhibitory activity of *F. velutipes* with incoherent light under the same conditions was 20–100%. As in case of *P. ostreatus* antimicrobial activity of

**Antimicrobial activity of macromycetes after light exposure**  
(diameters of zones of growth inhibition, mm)

Test-culture	Day of growth	Red		Green		Blue		Control without light exposure
		coherent	incoherent	coherent	incoherent	coherent	incoherent	
<i>P. ostreatus</i>								
<i>B. mycooides</i> 537	7	11 ± 0.16*	7 ± 0.2*	0	0	9 ± 0.14*	5 ± 0.23*	0
	14	19 ± 0.21*	14 ± 0.18*	(7 ± 0.16)*	(6 ± 0.14)*	14 ± 0.21*	12 ± 0.2*	5 ± 0.21
	21	0	18 ± 0.16*	(6 ± 0.21)*	(8 ± 0.14)*	19 ± 0.1*	17 ± 0.18*	13 ± 0.14
<i>B. pumilus</i> NCTC 8241	7	5 ± 0.02*	5 ± 0.26*	0	0	6 ± 0.12*	4 ± 0.1*	0
	14	15 ± 0.17*	16 ± 0.17*	9 ± 0.12*	6 ± 0.2*	12 ± 0.2*	10 ± 0.16*	8 ± 0.14
	21	0	0	0	0	5 ± 0.14	0	6 ± 0.17
<i>L. mesenteroides</i> VKPM B-4177	7	4 ± 0.12*	4 ± 0.10*	5 ± 0.33*	3 ± 0.10*	8 ± 0.22*	5 ± 0.1*	0
	14	10 ± 0.14*	9 ± 0.19*	10 ± 0.09*	6 ± 0.22*	10 ± 0.16*	6 ± 0.1*	5 ± 0.16
	21	8 ± 0.2*	6 ± 0.12*	0	0	0	3 ± 0.1*	5 ± 0.3
<i>S. aureus</i> FDA 209P (MSSA)	7	11 ± 0.2	10 ± 0.14*	8 ± 0.18*	7 ± 0.14*	12 ± 0.21*	10 ± 0.23*	5 ± 0.17
	14	16 ± 0.19*	13 ± 0.26*	8 ± 0.22	7 ± 0.18*	16 ± 1.7*	14 ± 0.23*	8 ± 0.24
	21	0	0	0	0	0	0	0
<i>S. aureus</i> INA 00761 (MRSA)	7	12 ± 0.16*	11 ± 0.2*	4 ± 0.12*	5 ± 0.1	14 ± 0.19*	11 ± 0.24*	5 ± 0.2
	14	20 ± 0.3*	18 ± 0.23*	11 ± 0.18*	9 ± 0.17*	19 ± 0.32*	16 ± 0.19*	10 ± 0.15
	21	0	0	(15 ± 0.33)*	(13 ± 0.22)*	0	0	16 ± 0.32
<i>M. luteus</i> NCTC 8340	7	0	0	0	0	0	0	0
	14	11 ± 0.1*	9 ± 0.22*	0	0	7 ± 0.11*	7 ± 0.2*	(6 ± 0.14)
	21	7 ± 0.17*	8 ± 0.12	0	0	11 ± 0.32*	9 ± 0.18*	(8 ± 0.16)
<i>E. coli</i> ATCC 25922	7	0	0	0	0	0	0	0
	14	12 ± 0.12*	10 ± 0.16*	9 ± 0.18*	7 ± 0.18*	13 ± 0.26*	10 ± 0.20*	6 ± 0.23
	21	15 ± 0.28*	13 ± 0.18*	12 ± 0.25*	10 ± 0.23*	16 ± 0.2*	13 ± 0.16*	9 ± 0.2
<i>C. terrigena</i> VKPM B-7571	7	10 ± 0.19*	9 ± 0.14*	0	0	10 ± 0.15*	12 ± 0.1*	0
	14	16 ± 0.26*	12 ± 0.17*	7 ± 0.12	7 ± 0.1	18 ± 0.3*	16 ± 0.18*	7 ± 0.12
	21	4 ± 0.23*	6 ± 0.23M	9 ± 0.24*	7 ± 0.1M	0	0	9 ± 0.12
<i>P. aeruginosa</i> ATCC 27853	7	0	0	0	0	0	0	0
	14	14 ± 0.17*	11 ± 0.18*	(8 ± 0.2)*	(9 ± 0.2)*	14 ± 0.16*	12 ± 0.23*	8 ± 0.21
	21	11 ± 0.16*	7 ± 0.25*	(7 ± 0.14)*	(7 ± 0.19)*	8 ± 0.18*	6 ± 0.21*	4 ± 0.17*
<i>F. velutipes</i>								
<i>B. mycooides</i> 537	7	6 ± 0.12*	5 ± 0.19*	0	0	9 ± 0.18*	3 ± 0.1*	0
	14	15 ± 0.22*	13 ± 0.1*	0	0	14 ± 0.11*	12 ± 0.18*	8 ± 0.23
	21	5 ± 0.2*	5 ± 0.23*	6 ± 0.12*	5 ± 0.2*	6 ± 0.14*	16 ± 0.17*	12 ± 0.32
<i>S. aureus</i> FDA 209P (MSSA)	7	13 ± 0.11*	9 ± 0.16*	0	0	10 ± 0.19*	6 ± 0.31*	0
	14	26 ± 0.3*	24 ± 0.23*	12 ± 0.27	11 ± 0.2*	28 ± 0.25*	23 ± 0.17*	12 ± 0.19
	21	8 ± 0.21*	6 ± 0.15*	18 ± 0.14*	20 ± 0.33*	9 ± 0.25*	8 ± 0.24*	22 ± 0.23

Test-culture	Day of growth	Red		Green		Blue		Control without light exposure
		coherent	incoherent	coherent	incoherent	coherent	incoherent	
<i>S. aureus</i> INA 00761 (MRSA)	7	7 ± 0.1*	4 ± 0.1*	0	0	11 ± 0.36**	9 ± 0.13*	0
	14	16 ± 0.14*	14 ± 0.22*	6 ± 0.11*	6 ± 0.16*	18 ± 0.21*	14 ± 0.11*	9 ± 0.1
	21	12 ± 0.31*	12 ± 0.15*	10 ± 0.2*	9 ± 0.21*	9 ± 0.11*	5 ± 0.1*	14 ± 0.26
<i>E. coli</i> ATCC 25922	7	6 ± 0.13*	6 ± 0.2*	0	0	8 ± 0.19*	5 ± 0.17*	0
	14	20 ± 0.17*	18 ± 0.32*	9 ± 0.12*	8 ± 0.26*	22 ± 0.34*	19 ± 0.12*	12 ± 0.14
	21	14 ± 0.24*	10 ± 0.11*	15 ± 0.34*	11 ± 0.1*	9 ± 0.19*	8 ± 0.11*	17 ± 0.19
<i>A. niger</i> INA 00760	7	16 ± 0.11*	12 ± 0.19*	7 ± 0.1*	8 ± 0.15*	19 ± 0.3*	17 ± 0.31*	10 ± 0.12
	14	12 ± 0.29*	15 ± 0.12*	10 ± 0.16*	10 ± 0.22*	12 ± 0.25*	13 ± 0.27*	14 ± 0.21
	21	9 ± 0.12*	10 ± 0.14*	8 ± 0.25*	7 ± 0.2*	6 ± 0.32*	5 ± 0.11*	12 ± 0.2
<b><i>G. applanatum</i></b>								
<i>B. subtilis</i> ATCC 6633	7	5 ± 0.11*	0	0	0	7 ± 0.16*	4 ± 0.11*	0
	14	13 ± 0.22*	11 ± 0.16*	8 ± 0.11	9 ± 0.28*	15 ± 0.36*	12 ± 0.18*	8 ± 0.12
	21	6 ± 0.18*	0	0	0	5 ± 0.11*	0	0
<i>S. aureus</i> FDA 209P (MSSA)	7	7 ± 0.1*	5 ± 0.19*	0	0	8 ± 0.23*	6 ± 0.21*	0
	14	17 ± 0.33*	15 ± 0.34*	12 ± 0.22*	11 ± 0.12*	16 ± 0.17*	14 ± 0.3*	13 ± 0.7
	21	4 ± 0.12	0	0	0	6 ± 0.15*	0	0
<i>S. aureus</i> INA 00761 (MRSA)	7	9 ± 0.14*	7 ± 0.19*	0	0	10 ± 0.22*	8 ± 0.13*	0
	14	18 ± 0.29*	17 ± 0.24*	10 ± 0.13*	11 ± 0.2*	16 ± 0.32*	14 ± 0.16*	12 ± 0.11
	21	7 ± 0.2*	6 ± 0.21*	0	0	0	0	0
<b><i>G. lucidum</i></b>								
<i>B. subtilis</i> ATCC 6633	7	9 ± 0.22*	6 ± 0.16*	0	0	7 ± 0.11*	6 ± 0.18*	0
	14	18 ± 0.15*	16 ± 0.13*	12 ± 0.3*	13 ± 0.34	19 ± 0.28*	16 ± 0.26*	13 ± 0.3
	21	6 ± 0.1*	0	0	0	4 ± 0.12*	0	0
<i>S. aureus</i> FDA 209P (MSSA)	7	5 ± 0.1*	5 ± 0.11*	0	0	7 ± 0.1*	6 ± 0.11*	0
	14	12 ± 0.18*	10 ± 0.21*	6 ± 0.12*	8 ± 0.13	14 ± 0.14*	11 ± 0.2*	8 ± 0.15
	21	0	0	0	0	0	0	0
<i>S. aureus</i> INA 00761 (MRSA)	7	6 ± 0.12*	0	0	0	4 ± 0.13*	0	0
	14	11 ± 0.2*	10 ± 0.19*	8 ± 0.14	7 ± 0.2*	12 ± 0.21*	9 ± 0.15*	8 ± 0.1
	21	0	0	0	0	0	0	0

Note: \*  $P < 0.05$ ; in particular pairs 5 and 6, 11 and 12 were statistically significant. For example,  $6 \pm 0,12$  and  $5 \pm 0,3$ : the value of the Student's t-test = 3.09, the differences were statistically significant ( $P < 0.05$ ), the number of degrees of freedom  $f = 4$ ; the critical value of the Student's t-test = 2.776, at a significance level  $\alpha = 0,05$ ;  $11 \pm 0,2$  and  $12 \pm 0,11$ : the value of the Student's t-test = 4.38; differences are statistically significant ( $P < 0.05$ ); the degrees of freedom  $f = 4$ ; the critical value of the Student's t-test = 2.776, at a significance level  $\alpha = 0,05$  (the same applies to the other pairs).

Control is everywhere; «0» — zero activity. In parentheses are the diameters of the zones of growth inhibition.

*F. velutipes* was observed a week earlier than in the control. Irradiation with green light inhibited the activity of strain *F. velutipes*. Similar changes of antimicrobial activity were observed after irradiation of strains *G. lucidum* and *G. applanatum*. Antimicrobial activity of these strains against methicillin-sensitive and methicillin-resistant strains of *S. aureus* and *B. subtilis* ATCC 6633 was previously identified [6]. However, unlike the previously described species these strains were active within a narrow time interval. Short-term exposure with low-intensity light in the blue and red wavelengths not only increased the antimicrobial activity of *G. lucidum* and *G. applanatum*, but also extended their biosynthetic activity. Perhaps these changes should be seen as positive, as some researchers think that for the antibiotic formation the long-term surface cultivation or submerged cultivation is needed [20, 21]. However, to prove this, additional accurate quantitative investigations of biosynthesis antibiotic components throughout the entire process of cultivation have to be performed. It should be noted that the mechanisms responsible for the biosynthesis of antimicrobial compounds from *G. lucidum* and *G. applanatum* were less sensitive to light exposure under proposed conditions. Maximal increasing of antimicrobial activity of *G. applanatum* against *B. subtilis* was observed when this fungi was irradiated with coherent red light and blue light (82 and 87%, respectively as compared with the non-irradiated control).

In case of *G. lucidum* the exposure under same conditions largely increased the inhibitory activity of the culture fluid against MSSA (50% — red and 75% — blue light). Green light did not have any significant effect on the duration of the biosynthesis or the level of the activity of antimicrobial components of these strains. Analysis of the results revealed some general patterns in the changes of antimicrobial dependence of the macromycete studied species after light exposure.

The stimulating effect of blue and red light let us suggest the presence of two photoreceptor systems that is in accordance to the modern theory of the universality of the mechanisms of photosensitivity [18]. There are several types of photoreceptors in fungi [14, 15, 19]. Isolation and characterization of fungi photoreceptors are based on the identified genes of WC-1 and WC-2 from *Neurospora crassa*. Genes which are similar to WC-1 and WC-2 have been identified in the genomes of some Ascomycetes, Basidiomycetes

and Zygomycetes, and many of these genes are required for the light photoresponse of fungi. The blue light was the most efficient from the point of view of impact on the mushroom photomorphogenesis. This light can also activate metabolism or affect the growth of fungal structures. Genes which are responsible for photoreception of blue light were found in basidiomycetes *Coprinus cinereus* (Schaeff.) Gray, *Pleurotus ostreatus* (Jacq.) P. Kumm. and *Lentinus edodes* (Berk.) Singer [22, 23]. Besides, research of fungi genome allowed to identify unexpected photoreceptor genes of phytochromes which are in particular sensitive to red light in addition to cryptochrome and rhodopsin which absorb the blue light [22, 24].

In all experiments macromycetes showed a higher sensitivity to low-intensity laser radiation. Experiments with different biological objects identified the highest bioactivity of coherent light compared to incoherent light at the same wavelength ranges by the spatial heterogeneity of the laser field. It is believed that the effectiveness of the coherent light is related to the influence of spatially heterogeneous distribution of the intensity of scattered coherent light [15, 18, 25], and the ability to create a high spectral radiation brightness, which can't be achieved by using conventional incoherent light sources.

It was found that low intensity light with the energy density of 230 mJ/cm<sup>2</sup> resulting from laser and LED light sources had a significant effect on the antimicrobial activity of *F. velutipes*, *P. ostreatus*, *G. lucidum* and *G. applanatum* at submerged cultivation. Short-term exposure of seed mycelium with red and blue wavelengths helped to reduce the period of cultivation and promoted the appearance of antimicrobial activity. Coherent (laser) light had a great stimulating effect as compared with incoherent (LED) and its usage under given conditions induced increase of the inhibitory activity of the culture fluid against different test-cultures: *F. velutipes* for 60–150%, *P. ostreatus* — 100–238%, *G. applanatum* — 30–87% and *G. lucidum* — 30–70%. However, the usage of LED sources provided quite high antimicrobial activity of *P. ostreatus* (37–180%) and *F. velutipes* (20–100%).

Thus, these results demonstrate the promising use of low-intensity light as an environmentally friendly stimulator of antimicrobial activity in biotechnology of submerged cultivation of macromycetes. The selected conditions of photostimulation may be used to intensify the output stages and to increase the yield of the final product.

## REFERENCES

- Arias C. A., Murray B. E., Murray B. E. Antibiotic-Resistant Bugs in the 21<sup>st</sup> Century — A Clinical Super-Challenge. *N. Engl. J. Med.* 2009, 360 (5), 439–443. doi:10.1056/NEJMp0804651.
- Sidorenko S. C. Rational antibiotic therapy and evidence based medicine. *Antibiotiki i Khimioterapiya*. 2001, N 9, P. 12–18. (In Russian).
- Donadio S., Carrano L., Brandi L., Serina S., Soffientini A., Raimondi E. Target sands says for discovering novel antibacterial agents. *J. Biotechnol.* 2002, V. 99, P. 175–185.
- Shivrina A. N., Nizkovskaya O. P., Falina N. N., Matisson N. L., Efimenko O. M. Biosynthetic activity of higher fungi. *Leningrad.: Nauka*. 1969, 243 p. (In Russian).
- Dyakov M. Ju., Kamzolkin O. V., Poedinok N. L., Bisko N. A., Mihaylova O. B., Efremenkova O. V. Morphological characteristics of natural strains of some macromycetes species and biological analysis of antimicrobial activity in submerged culture. *Mikol. Fitopatol.* 2010, 44 (3), 225–240. (In Russian).
- Krupodorova T. A., Bisko N. A., Poyedynok N. L., Mytropol'skaya N. Ju., Vasyleva B. F., Efremenkova O. V. Antimicrobial activity of strains of *Ganoderma applanatum* (Pers.: Wallr.) Pat and *G. lucidum* (Curt.: Fr.) P. Karst. under conditions of submerged cultivation. *Ukr. bot. zh.* 2008, V. 6, P. 590–594. (In Ukrainian).
- Alves M. J., Ferreira I. C., Dias J., Teixeira V., Martins A., Pintado M. A review on antimicrobial activity of mushroom (Basidiomycetes) extracts and isolated compounds. *Planta Med.* 2012, 78 (16), 1707–1718.
- Alves M. J., Ferreira I. C., Dias J., Teixeira V., Martins A., Pintado M. A. Review on antifungal activity of mushroom (basidiomycetes) extracts and isolated compounds. *Cur. Top. Med. Chem.* 2013, 13 (21), 2648–2659.
- Nagaraj K., Mallikarjun N., Naika R., Venugopal T. M. Phytochemical Analysis and In Vitro Antimicrobial Potential of *Ganoderma applanatum* (Pers.) Pat. of Shivamogga District-Karnataka, India. *Int. J. Pharm. Sci. Rev. Res.* 2013, 23 (2), 36–41.
- Ramesh Ch., M. G. Pattar M. G. Antimicrobial properties, antioxidant activity and bioactive compounds from six wild edible mushrooms of western ghats of Karnataka, India. *Pharm. Res.* 2010, 2 (2), 107–112. doi: 10.4103/0974-8490.62953.
- Ranadive K. R., Belsare M. H., Deokule S. S., Jagtap N. V., Jadhav H. K., Vaidya J. G. Glimpses of antimicrobial activity of fungi from World. *J. New Biologic. Reports*. 2013, 2 (2), 142–162.
- Oyetayo V. O., Ariyo O. O. Antimicrobial and Antioxidant Properties of *Pleurotus ostreatus* (Jacq: Fries) Cultivated on Different Tropical Woody Substrates. *J. Waste Convers. Bioprod. Biotechnol.* 2013, 1 (2), 28–32.
- Vamanu E. Studies on the antioxidant and antimicrobial activities of *Pleurotus ostreatus* PSI101109 mycelium. *Pakist. J. Bot.* 2013, 45 (1), 311–317.
- Corrochano L. M. Fungal photobiology: a synopsis. *IMA Fungus*. 2011, 2 (1), 25–28.
- Herrera-Estrella A., Horwitz B. A. Looking through the eyes of fungi: molecular genetics of photoreception. *Mol. Microbiol.* 2007, 64 (1), 5–15.
- Purschwitz J., Muller S., Kastner Ch., Fischer R. Seeing the rainbow: light sensing in fungi. *Cur. Opin. Microbiol.* 2006, 9 (6), 566–571.
- Poedinok N. L. Artificial light using in mushroom cultivation. *Biotechnologia Acta*. 2013, 6 (6), 58–70. (In Ukrainian).
- Karu T. J. Universal cellular mechanism of laser biostimulation: photoactivation of respiratory chain enzyme cytochrome oxidase. Holography: basic research, innovative projects and nanotechnology. *Materials XXVI School of coherent optics and holography. Irkutsk: "Papyrus"*. 2008, P. 156–175. (In Russian).
- Tisch D., Schmoll M. Light regulation of metabolic pathways in fungi. *Appl. Microbiol. Biotechnol.* 2010, 85 (5), 1259–1277.
- Moore D., Robson G. D., Trinci A. P. J. 21st Century Guidebook to Fungi. *Cambridge, UK: Cambridge University Press*. 2011, 236 p.
- Semerdzieva M., Veselsky J. Léčivé houby dřívě a nyní. *Praha: Academia*. 1986, 177 p.
- Kamada T., Sano H., Nakazawa T., Nakahori K. Regulation of fruiting body photomorphogenesis in *Coprinopsis cinerea*. *Fungal Gen. Biol.* 2010, 47 (11), 917–921.
- Nakano Y., Fujii N., Kojima M. Identification of Blue-Light Photoresponse Genes in Mushroom Mycelia. *Biosc. Biotechnol. Biochem.* 2010, 74 (10), 2160–2165.
- Galagan G. V., Calvo S. E., Borkovich K. A., Selker E. U., Read N. D., Jaffe D., FitzHugh W., Ma L. J., Smirnov S., Purcell S., Rehman B., Elkins T., Engels R., Wang S., Nielsen C. B., Butler J., Endrizzi M., Qui D., Ianakiev P., Bell-Pedersen D., Nelson M. A., Werner-Washburne M., Selitrennikoff C. P., Kinsey J. A., Braun E. L., Zelter A., Schulte U., Kothe G. O., Jedd G., Mewes W., Staben C., Marcotte E., Greenberg D., Roy A., Foley K., Naylor J., Stange-Thomann N., Barrett R., Gnerre S., Kamal M., Kamvysselis M., Mauceli E., Bielke C., Rudd S., Friselman D., Krystofova S., Rasmussen C., Metzenberg R. L., Perkins D. D., Kroken S., Cogoni C., Macino G., Catcheside D., Li W., Pratt R. J., Osmani S. A., DeSouza C. P., Glass L., Orbach M. J., Berglund J. A., Voelker R., Yarden O., Plamann M., Seiler S., Dunlap J., Radford A., Aramayo R., Natvig D. O., Alex L. A., Mannhaupt G., Ebbole D. J., Freitag M., Paulsen I., Sachs M. S., Lander E. S., Nusbaum C., Birren B. The genome sequence of the filamentous fungus *Neurospora crassa*. *Nature*. 2003, V. 422, P. 859–868.
- Zalevsky Z., Belkin M. Coherence and Speckle in Photomedicine and Photobiology. *Photomed. Laser Surgery*. 2011, 29 (10), 655–656.

## ІНДУКУВАННЯ АНТИМІКРОБНОЇ АКТИВНОСТІ ДЕЯКИХ МАКРОМИЦЕТІВ СВІТЛОМ НИЗЬКОЇ ІНТЕНСИВНОСТІ

Н. Л. Поєдинок<sup>1</sup>  
 О. Б. Михайлова<sup>2</sup>  
 А. М. Негрійко<sup>3</sup>  
 І. О. Дудка<sup>2</sup>  
 Б. Ф. Васильєва<sup>4</sup>  
 О. В. Єфременкова<sup>4</sup>

<sup>1</sup>ДУ «Інститут харчової біотехнології і геноміки НАН України», Київ

<sup>2</sup>Інститут ботаніки ім. М. Г. Холодного НАН України, Київ

<sup>3</sup>Інститут фізики НАН України, Київ

<sup>4</sup>Інститут з пошуку нових антибіотиків ім. Г. Ф. Гаузе РАМН, Москва, РФ

E-mail: poyedinok@uker.net

Метою роботи було вивчення індукування антимікробної активності макромицетів низькоінтенсивним світлом різної когерентності й різних довжин хвиль. Об'єктами дослідження були штами *Flammulina velutipes* 3923, *Pleurotus ostreatus* 531, *Ganoderma lucidum* 1908 і *G. applanatum* 1552 з Колекції культур шапинкових грибів Інституту ботаніки ім. М. Г. Холодного НАН України та тест-культури із Всеросійської колекції мікроорганізмів. Як джерело когерентного видимого світла використовували газові лазери: гелієво-неоновий лазер з випромінюванням на довжині хвилі 632,8 нм і аргонний іонний лазер — 488,0 і 514,5 нм. Для одержання некогерентного світла застосовували світлодіоди з випромінюванням на довжині хвиль 490,0, 520,0 і 634 нм. З'ясовано, що короткочасне опромінення посівного міцелію світлом низької інтенсивності зі щільністю енергії 230 мДж/см<sup>2</sup> у червоному і синьому діапазонах довжин хвиль скорочує період культивування до появи антимікробної активності та індукує збільшення інгібуючої активності культуральної рідини стосовно різних тест-культур від 20 до 238%. Встановлені режими фотостимуляції антимікробної активності можуть бути використані в біотехнології глибинного культивування макромицетів для інтенсифікації етапів і збільшення виходу кінцевого продукту.

**Ключові слова:** макромицети, низькоінтенсивне світло, лазери, антимікробна активність, світлодіоди.

## ИНДУЦИРОВАНИЕ АНТИМИКРОБНОЙ АКТИВНОСТИ НЕКОТОРЫХ МАКРОМИЦЕТОВ СВЕТОМ НИЗКОЙ ИНТЕНСИВНОСТИ

Н. Л. Поєдинок<sup>1</sup>  
 О. Б. Михайлова<sup>2</sup>  
 А. М. Негрійко<sup>3</sup>  
 И. А. Дудка<sup>2</sup>  
 Б. Ф. Васильєва<sup>4</sup>  
 О. В. Ефременкова<sup>4</sup>

<sup>1</sup>ДУ «Институт пищевой биотехнологии и геномики НАН Украины», Киев

<sup>2</sup>Институт ботаники им. Н. Г. Холодного НАН Украины, Киев

<sup>3</sup>Институт физики НАН Украины, Киев

<sup>4</sup>Институт по изысканию новых антибиотиков им. Г. Ф. Гаузе РАМН, Москва, РФ

E-mail: poyedinok@uker.net

Целью работы было изучение индуцирования антимикробной активности макромицетов низкоинтенсивным светом разной когерентности и разных длин волн. Объектами исследования были штаммы *Flammulina velutipes* 3923, *Pleurotus ostreatus* 531, *Ganoderma lucidum* 1908 и *G. applanatum* 1552 из Коллекции культур шляпочных грибов Института ботаники им. Н. Г. Холодного НАН Украины и тест-культуры из Всеросийской коллекции микроорганизмов. В качестве источника когерентного видимого света использовали газовые лазеры: гелиево-неоновый лазер с излучением на длине волны 632,8 нм и аргонный ионный лазер — 488,0 и 514,5 нм. Для получения некогерентного света применяли светодиоды с излучением на длине волн 490,0, 520,0 и 634,0 нм. Установлено, что кратковременное облучение посевного мицелия светом низкой интенсивности с плотностью энергии 230 мДж/см<sup>2</sup> в красном и синем диапазонах длин волн сокращает период культивирования до появления антимикробной активности и индуцирует увеличение ингибирующей активности культуральной жидкости по отношению к разным тест-культурам от 20 до 238%. Установленные режимы фотостимуляции антимикробной активности могут быть использованы в биотехнологии глубинного культивирования макромицетов для интенсификации этапов и увеличения выхода конечного продукта.

**Ключевые слова:** макромицеты, низкоинтенсивный свет, лазеры, антимикробная активность, светодиоды.