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ANALYSIS OF THE INFLUENCE OF LASER IRRADIATION ON THE ACCUMULATION OF BIOMASS AND POLYSACCHARIDES PLEUROTUS OSTREATUS (JACQ.) P. KUMM

K. Reshetnyk

The aim of the study *was to investigate the effect of laser irradiation of mycelium on the amount of biomass and synthesis of P. ostreatus polysaccharides.*
Materials and methods. *For the study, 6 strains of P. ostreatus from the Collection of cultures of basidiomycetes of the Department of Botany and Ecology of Vasyl Stus DonNU were used. A device consisting of LED lasers was used for laser irradiation of vegetative mycelium: BRP – 3010–5, with red spectrum radiation with a wavelength of 635 nm; BBP – 3010–5 with blue spectrum radiation with a wavelength of 405 nm and BGP – 3010–5 with green spectrum radiation with a wavelength of 532 nm. The level of biomass accumulation was determined by weight. The polysaccharide content was determined by the phenol-sulfur method.*
Results. *The most effective was green light irradiation with a wavelength of 532 nm. For strain P-192 – the amount of biomass increased by 71.4 %. For strains P-191 and P-155 biomass increased by 60 % and 53.5 %. For strains P-108, P-154 and P-6v, the amount of biomass increased from 33.3 to 50 %. For strain P-192, the amount of mycelial endopolysaccharides increased by 42.0 %. For strains P-191 and P-6v, the amount of endopolysaccharides increased by 39.3 % and 38.7 %. For strains P-108, P-155 and P-154 the amount of mycelium endopolysaccharides increased from 30.7 % to 35.8. For strain P-192 the content of exopolysaccharides increased by 30.5 %. For strains P-154 and P-191, the amount of exopolysaccharides increased by 28.1 % and 27.8 %. For strains P-108, P-155 and P-6v the content of exopolysaccharides increased from 24.6 % to 25.8 %.*
Conclusions. *The most effective mode of photoactivation of P. ostreatus mycelium for obtaining target products was determined. In particular, the best response was observed in response to green light with a wavelength of 532 nm for strain P-192 – the amount of biomass increased by 71.4 %, the amount of mycelium endopolysaccharides increased by 42.0 %, and the content of exopolysaccharides increased by 30.5 %*
Keywords: *Pleurotus ostreatus, laser irradiation, surface cultivation, photoactivation, vegetative mycelium, polysaccharides*

1. Introduction

Pleurotus ostreatus belongs to the fungi that cause white rot of wood and has a well-developed enzymatic apparatus that is able to absorb a variety of carbon-containing substrates [1, 2]. *P. ostreatus* contains a significant amount of protein, polysaccharides and other biologically active substances (BAS), which have antimicrobial, antiviral, antitumor, antioxidant, hyperglycemic, anti-inflammatory, hepatoprotective, hypocholesterolemic, immunomodulatory properties [3]. The prospects of its study are also due to the fact that in terms of production in Ukraine it ranks second place, and industrial cultivation of this fungus is 16.3 % of total world production [4]. *P. ostreatus* attracts the attention of scientists due to its accessibility and ease of use. This allows the introduction of new modern methods of intensification of growth and biochemical parameters of this fungus. In particular, this method is laser irradiation of the mycelium, which can significantly improve the growth and development of fungi. It should be noted that the use of helium-neon and argon lasers, which have large dimensions and significant energy consumption, complicates the technology of stimulating the growth and development of fungi [5]. In our opinion, it is much more effective to use LED lasers to intensify the metabolic processes of macromycetes [6].

2. Literary review

From the literature it is known that the cultivation of mycelium of *P. ostreatus* is more economical and takes less time compared to growing fruit bodies. Scientists pay special attention to obtaining biomass [4] and polysaccharides of *P. ostreatus* [7, 8]. The influence of substrates from extracts of waste sunflower husk, oat seed meal, milk thistle, flax, pumpkin, mustard, rose hips, wheat germ, amaranth seed meal, rapeseed meal, sunflower, ryegrass, walnut, yam, sweet and ordinary potato, cattail rhizome, plantain on biomass characteristics and oyster fungus metabolites [7, 9]. The stimulating effect of yam extract on the accumulation of *P. ostreatus* biomass and exopolysaccharides has been proved [7, 9]. It is known that the use of a mixture of winemaking waste and wheat bran gave a high yield of *P. ostreatus* biomass [10]. Irradiation of sowing mycelium of *G. lucidum* and *L. edodes* with both coherent and incoherent light of low intensity, where as a source of coherent visible light used modifications of gas lasers: helium-neon LGN-215 with radiation at a wavelength of 632.8 nm

(red light) and argon ion laser (modified model LGN-106M1) - radiation with a wavelength of 514.5 (green light) and 488.0 nm (blue light) caused an increase in polysaccharide synthesis and the amount of biomass [11]. In previous studies, we found that laser irradiation of vegetative mycelium of *P. ostreatus* with light of different spectral composition activates mycelial growth, so we hypothesized that the use of photoactivated mycelium will increase the synthesis of biologically active substances and the amount of biomass [6].

3. The purpose and objectives of the study

The aim of the study was to study the effect of laser irradiation of mycelium on the amount of biomass and synthesis of *P. ostreatus* polysaccharides.

To solve this purpose, the following tasks were solved:

1. To establish the effect of laser irradiation with light of different spectral composition on the amount of biomass of the fungus *P. ostreatus*.

2. To investigate the effect of laser irradiation on the synthesis of polysaccharides of the fungus *P. ostreatus*.

3. Determine the most effective mode of photoactivation of *P. ostreatus* mycelium to obtain target products.

4. Materials and methods of research

The research was conducted at the Department of Botany and Ecology of Vasyl Stus Donetsk National University (Vasyl Stus DonNU). Six strains of the fungus *P. ostreatus* from the Collection of cultures of basidiomycetes of the Department of Botany and Ecology of Vasyl Stus DonNU, which are part of the Basidiomycota department, were used for the study. To obtain the inoculum of mycelium strains P-191, P-192, P-6v, P-154, P-155, P-108 of the fungus *P. ostreatus* was cultured on agar glucose-peptone medium (GPA), g / l: glucose – 10.0; peptone – 3.0; KH_2PO_4 – 0.6; K_2HPO_4 – 0.4; $MgSO_4\,7{\cdot}H_2O$ – 0.5; $CaCl_2$ – 0.05; $ZnSO_4{\cdot}7\,H_2O$ – 0.001, distilled water – up to 1 dm^3; agar-agar – 15 in Petri dishes at a temperature of 26±1 °C. In the study of the effect of laser irradiation of the mycelium in the surface culture used glucose-peptone liquid nutrient medium (GPM), g/l: glucose – 10.0; peptone – 3.0; KH_2PO_4 – 0.6; K_2HPO_4 – 0.4; $MgSO_4\,7{\cdot}H_2O$ – 0.5; $CaCl_2$ – 0.05; $ZnSO_4{\cdot}7\,H_2O$ – 0.001, distilled water – up to 1 dm^3. The mycelium was cultured for 12 days at 26±1 °C in a thermostat in 250 ml Erlenmeyer flasks containing 50 ml of GPM nutrient medium. Inoculation was performed with disks of mycelium of cultures grown on GPA. Five disks with a diameter of 5 mm were cut with a sterile steel tube at a distance of 8–10 mm from the edge of the active growth of the colony.

For laser irradiation of the vegetative mycelium, a device consisting of an octagonal mirror prism that perceives the beam of LED lasers was used: BRP – 3010–5, with red spectrum radiation with a wavelength of 635 nm; BBP – 3010–5 with blue spectrum radiation with a wavelength of 405 nm and BGP – 3010–5 with green spectrum radiation with a wavelength of 532 nm (laser manufacturer BOB LASER Co., China) and reflects it on the conveyor belt on which the cup is placed Petri dish with mycelium. The power of each laser is - 100 mW. The device has two electric motors that are responsible for the movement of the mirror prism and the conveyor belt. The device is controlled by a control panel equipped with buttons to adjust the exposure time and select the desired laser with the appropriate wavelength of light (Fig. 1).

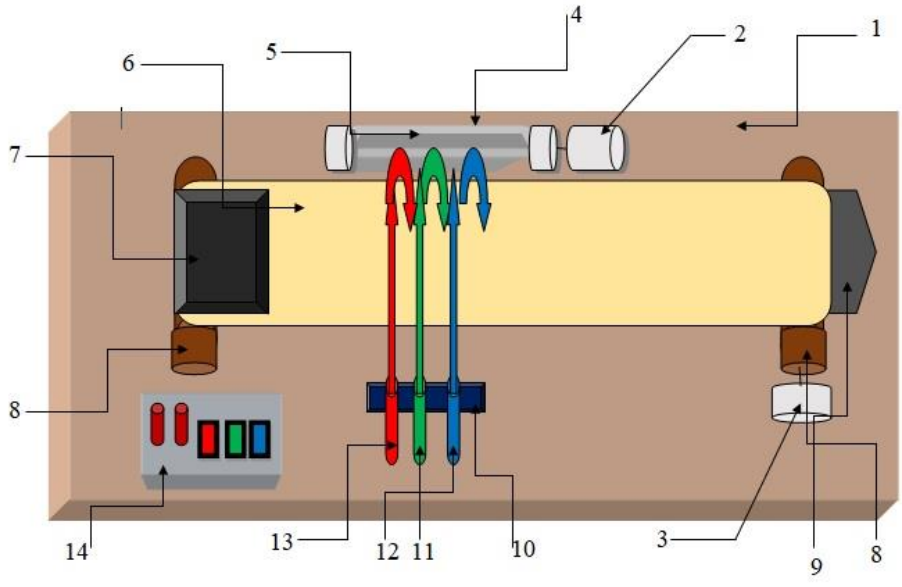

Fig. 1. Device for irradiating mycelium with monochromatic light using LED lasers: 1 – platform for mounting the device, 2 – electric motor 1, 3 – electric motor 2, 4 – protective cover for a mirror prism, 5 – mirror prism, 6 – conveyor belt, 7 – hopper for irradiated objects, 8 – the roller that moves the conveyor belt, 9 – platform for irradiated objects, 10 – tripod for mounting LED lasers, 11 – LED laser BGP-3010-5 with green spectrum radiation with a wavelength of 532 nm, 12 – LED laser BBP-3010-5 with blue spectrum radiation with a wavelength of 405 nm, 13 – LED laser BRP-3010-5, with red spectrum radiation with a wavelength of 635 nm, 14 – control panel

The mycelium was irradiated as follows: a Petri dish with mycelium moves on a conveyor belt under a beam of light with a set wavelength: 635, 405 and 532 nm, obtaining the required irradiation energy (51.1 mJ / cm^2). Irradiation of the mycelium lasted 10 s. Then, using a sterile steel tube, 5 mm diameter mycelial discs were excised from the mycelial colony and inoculated onto a liquid nutrient medium (GPM) of appropriate composition.

An irradiated culture was used to inoculate the control Petri dishes. Irradiation of the mycelium was performed in several variants (Table 1).

Table 1

Scheme of irradiation of the mycelium of the *Pleurotus ostreatus* fungus

Irradiation option	Duration of irradiation, p			Irradiation energy, mJ /cm^2
	Red light (wavelength 635 nm)	Blue light (wavelength 405 nm)	Green light (wavelength 532 nm)	
1 (control)	0	0	0	0
2	10	0	0	51.1
3	0	10	0	51.1
4	0	0	10	51.1

The level of biomass accumulation was determined by the weight method, drying the mycelium to a constant mass at a temperature of (105±1) °C [12].

To quantify the endopolysaccharides, a crushed 100 mg portion of dry mycelium was taken, transferred to a 20 ml tube, 5 ml of 1M NaOH was added, capped and extracted in a thermostat at 60 °C for 1 h, stirring occasionally. The resulting extract was centrifuged for 20 min at 6000 rpm. The precipitate was separated, the content of endopolysaccharides in the supernatant was determined by phenol-sulfur method [13].

To determine the concentration of exopolysaccharides first carried out the precipitation of 5 ml of culture fluid 10 ml of 96 % ethanol and settling during the day at (4±1) °C, after which the precipitate was separated by centrifugation for 25 minutes at 6000 rpm, dissolved in 5 ml of hot distilled water and took 2 ml of a solution in which the amount of exopolysaccharides was determined by the phenol-sulfur method [13].

All experiments were performed in triplicate. To determine the probability of the effect of laser radiation on the amount of biomass and polysaccharides used the method of analysis of variance. Comparison of mean values was performed by the Dunnett method [14]. Processing was performed using a package of statistical programs created at the Department of Plant Physiology and Biochemistry of DonNU named after Vasyl Stus [15].

5. Research results and their discussion

Analysis of the results of our studies shows a positive effect of radiation on the accumulation of biomass by the studied strains of the fungus *P. ostreatus*. In particular, for *P. ostreatus*, green light irradiation with a wavelength of 532 nm (irradiation energy 51.1 mJ/cm^2) was the most effective. Under the action of this irradiation regime, the best response to light was observed for strain P-192 – the amount of biomass increased by 71.4 % according to the control. For strains R-191 and R-155 biomass increased by 60 % and 53.5 %, respectively. For strains P-108, P-154 and P-6v, the amount of biomass increased from 33.3 to 50 %, respectively. Laser irradiation with red light with a wavelength of 635 nm (irradiation energy 51.1 mJ/cm^2) led to an increase in biomass for all studied strains in the range from 16 % to 25 %, and for strain P-155 this figure increased by 35.7 %. Irradiation of the mycelium with blue light with a wavelength of 405 nm (irradiation energy 51.1 mJ/cm^2) caused an increase in mycelial biomass for all studied strains of *P. ostreatus* in the range from 11.9 to 31.2 %, respectively (Fig. 2).

We first established the content of endopolysaccharides in the biomass of the fungus and exopolysaccharides in the culture fluid under the action of laser irradiation (Fig. 3). For strain P-192 *P. ostreatus*, the results were as follows: the most effective is green light irradiation with a wavelength of 532 nm (irradiation energy 51.1 mJ/cm^2). Under the action of this irradiation regime, the amount of mycelium endopolysaccharides increased by 42.0 % according to the control. For strains P-191 and P-6v, the amount of endopolysaccharides increased by 39.3 % and 38.7 %, respectively. For strains P-108, P-155 and P-154, the amount of mycelium endopolysaccharides increased from 30.7 % to 35.8, respectively. We also detected a mycelial reaction in response to the action of blue light with a wavelength of 405 nm (irradiation energy 51.1 mJ/cm^2). In particular, under this mode of photostimulation, the amount of mycelium endopolysaccharides increased from 12.5 % to 16.6 % for all studied strains. R-108, R-155 and R-154 from 15.3 % to 16.6 % respectively. Slightly lower values for this mode of irradiation were found for strains P-191, P-192 and P-6v – from 6.2 to 6.6 %, respectively (Fig. 3).

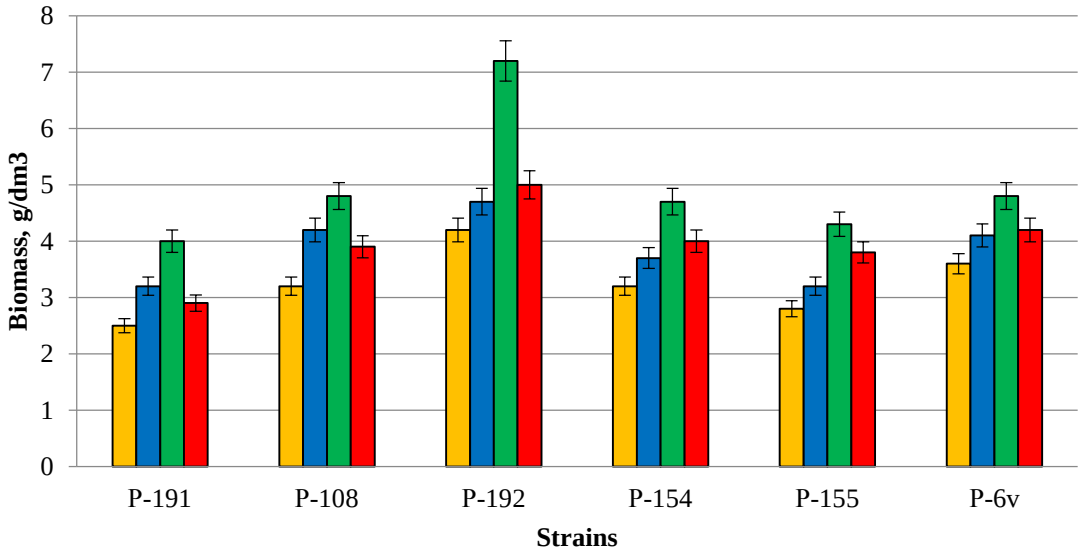

Fig. 2. The effect of laser irradiation on the amount of biomass of Pleurotus ostreatus strains when cultured on glucose-peptone medium. 12 days of cultivation: – without irradiation; – 405 nm; – 635 nm; – 532 nm

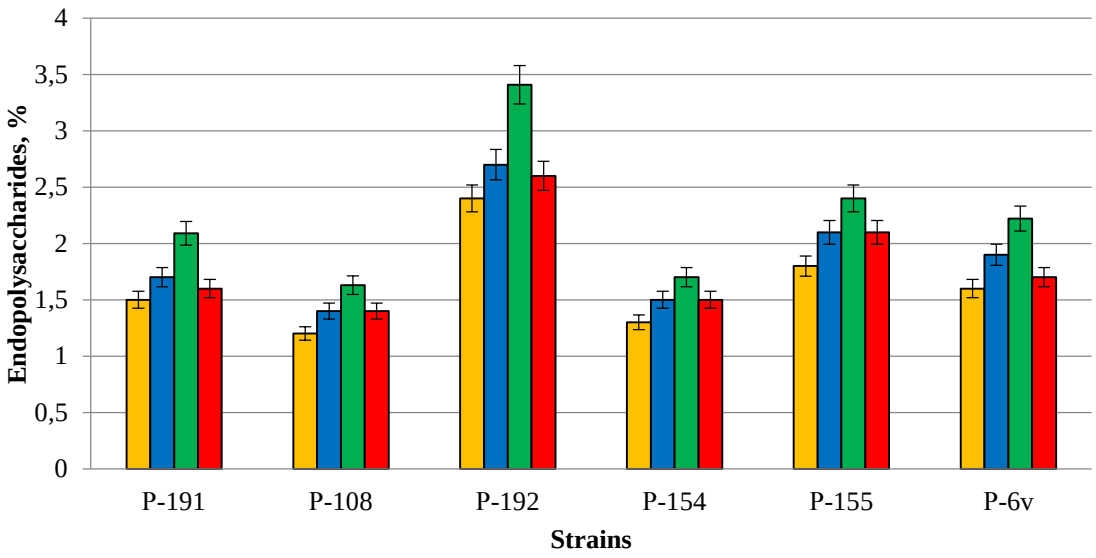

Fig. 3. The content of endopolysaccharides of Pleurotus ostreatus strains when cultured on glucose-peptone medium under the action of laser irradiation. 12 days of cultivation:
– without irradiation; – 405 nm; – 635 nm; – 532 nm

Data on the amount of exopolysaccharides were slightly lower and varied depending on the irradiation regime. For strain P-192 *P. ostreatus*, the most effective was irradiation with green light with a wavelength of 532 nm (irradiation energy 51.1 mJ/cm^2). Under the action of this irradiation regime, the content of exopolysaccharides increased by 30.5 % according to the control. For strains P-154 and P-191, the amount of exopolysaccharides increased by 28.1 % and 27.8 %, respectively. For strains P-108, P-155 and P-6v the content of exopolysaccharides increased from 24.6 % to 25.8 %, respectively. The amount of exopolysaccharides under the action of irradiation of mycelium with blue light at a wavelength of 405 nm (irradiation energy 51.1 mJ/cm^2) increased from 11.6 % to 20.0 % for all studied strains Laser irradiation of mycelium with red light at a wavelength of 635 nm (energy about 51.1 mJ/cm^2) caused an increase in the content of exopolysaccharides for all studied strains from 9.2 % to 16.6 %, respectively (Fig. 4).

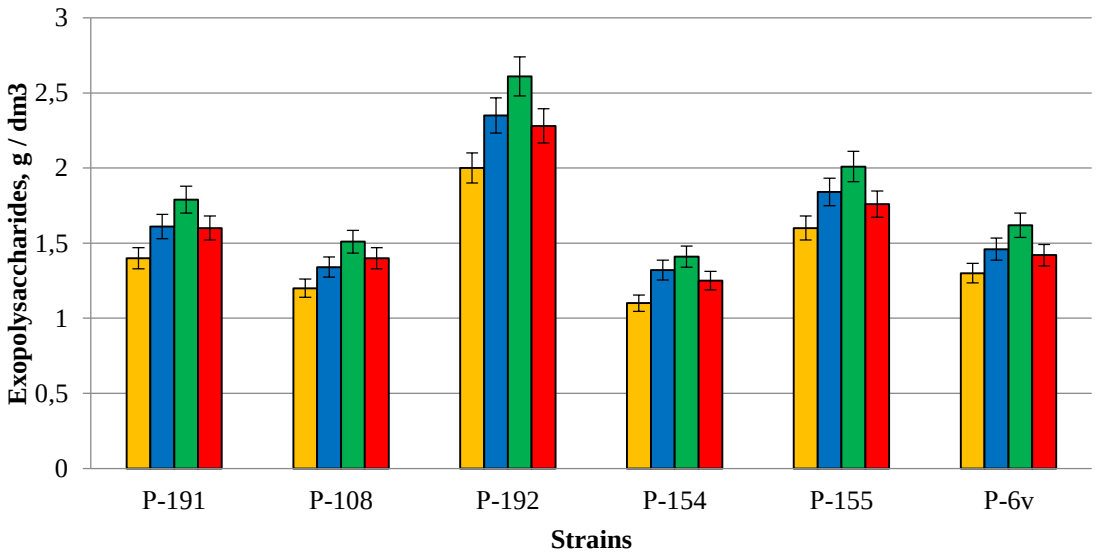

Fig. 4. The content of exopolysaccharides of Pleurotus ostreatus strains when cultured on glucose-peptone medium under the action of laser irradiation. 12 days of cultivation:
– without irradiation; – 405 nm; – 635 nm; – 532 nm

6. Research results discussion

It is known from the literature that the growth of biomass accumulation during cultivation varies depending on the strain and irradiation regime. In particular, for *F. velutipes* (coherent light with a wavelength of 488.0 nm) biomass increased by 34.2 %, for *P. ostreatus* (coherent light with a wavelength of 632.8 nm) by 41.8 % [5]. Structural polysaccharides and chitin are important components of the cell wall of fungi. The presence of polysaccharides in the biomass of *P. ostreatus* during the cultivation of mycelium on different substrates has been established [9]. Our data on the content of polysaccharides of *P. ostreatus* correspond to the literature [8, 16], but are slightly lower. In our opinion, this may be due to the composition of nutrient media used by other scientists, in particular the presence of complex organic components, such as oat seed meal, milk thistle, flax, pumpkin, mustard, rose hips, wheat germ, amaranth seed meal, cake from rapeseed, sunflower seeds, red rice, walnut, yam, sweet and ordinary potato, cattail rhizome, plantain, etc.

We have for the first time obtained interesting data on the effect of laser irradiation on the synthesis of biomass and polysaccharides of the fungus *P. ostreatus*. According to which there is a significant increase in the amount of biomass and synthesis of polysaccharides under the action of irradiation of the mycelium with green light with a wavelength of 532 nm for 10 s. The results obtained by us demonstrate the prospects of using laser irradiation for targeted regulation of biomass and polysaccharide synthesis.

Study limitations. It should be noted that this study was limited by the number of options used with a certain wavelength of light, as it requires re-equipment of the device. In addition, the data obtained reflect the reaction of strains only to specific light, which was used in our studies with a well-defined wavelength.

Prospects for further research. At the same time, we found differences in the photosensitivity of the studied strains of the *P. ostreatus* fungus depending on the wavelength of light makes it necessary to further search for more effective modes of photostimulation.

7. Conclusions

1. It was found that laser irradiation of mycelium with green (532 nm), blue (405 nm) and red (635 nm) light (irradiation energy 51.1 mJ / cm2) leads to an increase in the amount of mycelial biomass of the studied strains of *P. ostreatus*.

2. It was investigated that laser irradiation with green (wavelength 532 nm), blue (wavelength 405 nm) and red light with a wavelength of 635 nm (irradiation energy 51.1 mJ / cm^2) contributes to the increase of endopolysaccharides in biomass and exopolysaccharides in the culture fluid of the studied strains of *P. ostreatus*.

3. The most effective mode of photoactivation of *P. ostreatus* mycelium for obtaining target products was determined. In particular, the best response was observed in response to green light at 532 nm for strain P-192 – the amount of biomass increased by 71.4 %, the amount of mycelium endopolysaccharides increased by 42.0 %, and the content of exopolysaccharides increased by 30.5 %.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Kateryna Reshetnyk, Senior Lecturer, Department of Botany and ecology, Vasyl' Stus Donetsk National University, 600-richchia str., 21, Vinnitsa, Ukraine, 21021,
E-mail: k.reshetnyk@donnu.edu.ua