The influence of laser irradiation and glucose concentration on the content of carotenoids in the mycelium of fungus *Laetiporus sulphureus* (Bull.) Murrill

KATERYNA SERGIIVNA RESHETNYK

RESHETNYK K.S. (2020). The influence of laser irradiation and glucose concentration on the content of carotenoids in the mycelium of fungus *Laetiporus sulphureus* (Bull.) Murrill. *Chornomors'k. bot. z.*, **16** (4): 333–342. doi: 10.32999/ksu1990553X/2020-16-4-6

The article presents the results of the study of the content of carotenoids of L. sulphureus mycelium under the action of LED lasers: 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 (irradiation energy 51.1 mJ/cm²) when cultured on nutrient medium with different concentrations of glucose. The irradiated mycelium served as a control. It was found that is most effective for the synthesis of carotenoids the use of glucose-peptone medium with a glucose concentration of 10 g/dm³ in combination with irradiation of mycelium with green light at a wavelength of 532 nm (irradiation energy 51.1 mJ/cm²). Under the action of this irradiation regime for strain L.s.-18 the content of carotenoids in the mycelium increased by 66.1% according to the control. Laser irradiation of mycelium with blue light with a wavelength of 405 nm (irradiation energy 51.1 mJ/cm²) increased the content of carotenoids for strain L.s.-18 by 46.7%. Irradiation with red light with a wavelength of 635 nm (irradiation energy 51.1 mJ/cm²) contributed to an increase in the content of carotenoids for strain L.s.-16 of the fungus L. sulphureus by 28.9%. It was found that the use of glucose-peptone medium with a glucose concentration of 8 g/dm³ in combination with irradiation of the mycelium with green light with a wavelength of 532 nm (irradiation energy 51.1 mJ/cm²) was less effective. Under these conditions, the content of carotenoids in the mycelium increased for strain L.s.-17 by 62.3%. Laser irradiation of mycelium with blue light with a wavelength of 405 nm (irradiation energy 51.1 mJ/cm²) increased the content of carotenoids for strain L.s.-17 by 30.6%. Irradiation with red light with a wavelength of 635 nm (irradiation energy 51.1 mJ/cm²) contributed to an increase in the content of carotenoids for strain L.s.-18 of the fungus L. sulphureus by 16.8% respectively. For strain L.s.-16 the number of carotenoids in the mycelium no increase. The use glucosepeptone medium with glucose concentrations of 6 and 4 g/dm³ in combination with laser irradiation of mycelium with red (wavelength 635 nm), blue (wavelength 405 nm) and green (wavelength 532 nm) light with irradiation energy 51.1 mJ/cm² was no increase in the content of carotenoids in the mycelium.

Keywords: mycelium, carotenoids, photoreception, photoactivation

РЕШЕТНИК К.С. (2020). **Вплив лазерного випромінювання та концентарції глюкози на вміст каротиноїдів у міцелії грибів** *Laetiporus sulphureus* (**Bull.**) **Murrill.** *Чорноморськ. бот. ж.*, **16** (4): 333–342. doi: 10.32999/ksu1990553X/2020-16-4-6

У статті представлено результати дослідження кількості каротиноїдів міцелію *L. sulphureus* за дії LED лазерів: BRP–3010–5, з випромінюванням червоного спектру з довжиною хвилі 635 нм, BBP–3010–5 з випромінюванням синього спектру з довжиною хвилі 405 нм та BGP–3010–5 з випромінюванням зеленого спектру з довжиною хвилі 532 нм (енергія опромінення 51,1 мДж/см²) при культивуванні на живильному середовищі з різними концентраціями глюкози. Контролем слугував



© Reshetnyk K.S.

неопромінений міцелій. Встановлено, що найефективнішим для синтезу каротиноїдів є використання глюкозо-пептонного середовища з концентрацією глюкози 10 г/дм³ у комплексі з опроміненням міцелію зеленим світлом довжиною хвилі 532 нм (енергія опромінення 51,1 мДж/см²). За дії цього режиму опромінення для штаму L.s.-18 вміст каротиноїдів у міцелії зріс на 66,1 % відповідно до контролю. Лазерне опромінення міцелію синім світлом довжиною хвилі 405 нм (енергія опромінення 51,1 мДж/см²) збільшило кількість каротиноїдів для штаму L.s.-18 на 46,7 %. Опромінення червоним світлом довжиною хвилі 635 нм (енергія опромінення 51,1 мДж/см²) сприяло зростанню кількості каротиноїдів для штаму L.s.-16 гриба L. sulphureus на 28,9 %. Встановлено, що використання глюкозо-пептонного середовища з концентрацією глюкози 8 г/дм³ у комплексі з опроміненням міцелію зеленим світлом довжиною хвилі 532 нм (енергія опромінення 51,1 мДж/см²) було менш ефективним. За цих умов вміст каротиноїдів у міцелію зріс для штаму L.s.-17 на 62,3%. Лазерне опромінення міцелію синім світлом довжиною хвилі 405 нм (енергія опромінення 51,1 мДж/см2) збільшило кількість каротиноїдів для штаму L.s.-17 на 30,6% відповідно. Опромінення червоним світлом довжиною хвилі 635 нм (енергія опромінення 51,1 мДж/см²) сприяло зростанню кількості каротиноїдів для штаму Ls-18 гриба L. sulphureus на 16,8% відповідно. Для штаму L.s.-16 кількість каротиноїдів у міцелії не зросла. Під час використання глюкозо-пептонного середовища концентраціями глюкози 6 та 4 г/дм³ у комплексі з лазерним опромінення міцелію червоним (довжина хвилі 635 нм), синім (довжина хвилі 405 нм) та зеленим (довжина хвилі 532 нм) світлом з енергією опромінення 51,1 мДж/см² не відбувалося зростання вмісту каротиноїдів у міцелії.

Ключові слова: міцелій, каротиноїди, фоторецепція, фотоактивація

РЕШЕТНИК К.С. (2020). Влияние лазерного излучения и концентрации глюкозы на содержание каротиноидов в мицелии грибов *Laetiporus sulphureus* (Bull.) Murrill. *Черноморськ. бот. ж.*, **16** (4): 333–342. doi: 10.32999/ksu1990553X/2020-16-4-6

В статье представлены результаты исследования количества каротиноидов мицелия L. sulphureus за действия LED лазеров: BRP-3010-5, с излучением красного спектра с длиной волны 635 нм, ВВР-3010-5 с излучением синего спектра с длиной волны 405 нм и BGP-3010-5 с излучением зеленого спектра с длиной волны 532 нм (энергия облучения 51,1 мДж/см²) при культивировании на питательной среде с различными концентрациями глюкозы. Контролем служил необлученный мицелий. Установлено, что наиболее эффективным для синтеза каротиноидов является использование глюкозо-пептонного среды с концентрацией глюкозы 10 г/дм³ в комплексе с облучением мицелия зеленым светом длиной волны 532 нм (энергия облучения 51,1 мДж/см²). За действия этого режима облучения для штамма L.s.-18 содержание каротиноидов в мицелии выросло на 66,1% в соответствии с контроля. Лазерное облучение мицелия синим светом длиной волны 405 нм (энергия облучения 51,1 мДж/см²) увеличило количество каротиноидов для штамма L.s.-18 на 46,7% соответственно. Облучения красным светом длиной волны 635 нм (энергия облучения 51,1 мДж/см²) способствовало росту числа каротиноидов для штаму L.s.-16 гриба L. sulphureus на 28,9%. Установлено, что использование глюкозо-пептонной среды с концентрацией глюкозы 8 г/дм³ в комплексе с облучением мицелия зеленым светом длиной волны 532 нм (энергия облучения 51,1 мДж/см²) было менее эффективным. В этих условиях содержание каротиноидов в мицелия выросло для штамма L.s.-17 на 62,3%. Лазерное облучение мицелия синим светом длиной волны 405 нм (энергия облучения 51,1 мДж/см²) увеличило количество каротиноидов для штамма L.s.-17 на 30,6%. Облучения красным светом длиной волны 635 нм (энергия облучения 51,1 мДж/см²) способствовало росту числа каротиноидов для штамма L.s.-18 гриба L. sulphureus на 16,8% соответственно. Для штамма L.s.-16 количество каротиноидов в мицелии не выросло. При использовании глюкозо-пептонной среды с концентрациями глюкозы 6 и 4 г/дм³ в комплексе с лазерным облучения мицелия красным (длина волны 635 нм), синим (длина волны 405 нм) и зеленым (длина волны 532 нм) светом с энергией излучения 51,1 мДж/см² не происходило роста содержания каротиноидов в мицелии.

Ключевые слова: мицелий, каротиноиды, фоторецепция, фотоактивация

Carotenoids perform more than 20 biological functions – from photoreception to protecting the organism from lipid peroxidation [BRITTON, 1986]. From literary sources, it is known that fungi, in which the presence of mycochromic systems is established, have carotenoid pigments. Among them the fungus *Laetiporus sulphureus* (Bull.) Murrill., which is noted for a fairly high carotenoid content. These pigments are closely associated with the cell membrane and are able to reduction-oxidation (redox) reactions [ZHDANOVA, VASILEVSKAYA, 1982]. Carotenoids take part in protecting the body from the effects of adverse environmental factors, stabilize membranes, and are hormone precursors [KARNAUKHOV, 1986]. They play a role in the processes of differentiation and in the reactions of phototropism and phototaxis [GESSLER et al., 2002; 2006].

It is known that carotenoids have antioxidant, radioprotective, anticancer, immunomodulatory and other medicinal properties [BUTSENKO et al., 2010; GESSLER et al., 2003; GOODWIN, 1980]. Carotenoids are used as dyes and antioxidants in various fields industry [FEDOTOV, 2007; ELDAHSHAN et al., 2013]. Accordingly, a wide range of uses of these pigments requires the search for new organisms-producers to obtain them. One of such organisms that are able to synthesize carotenoids are fungi [BECKER, 1988; GOODWIN, 1980; RIBEIRO et al. 2011]. In particular, the biomass of the fungi Blakeslea trispora and Neurospora crassa is already used to obtain carotenoids [Becker, 1988; Gessler et al. 2003]. It was studied that the fruiting bodies of fungi of the genera Hygrophorus, Fistulina, Cantharellus, Boletus, Suillus also contain carotenoids [RIBEIRO et al., 2011]. Among the fungi, one of the most promising producers of carothenoids is the agaricomycet L. sulphureus. This fungus can be widely used for obtaining preparations possessing antioxidant protection [VELYGODSKAYA, FEDOTOV, 2016]. It is established that the intensity of metabolic processes in the fungal organism significantly depends on cultivation factors in ex-situ studies [BECKER, 1988]. Because carotenoids are secondary metabolites, there is a possibility of regulation their synthesis by changing the conditions of cultivation of producer strains, including and the composition of nutrient media [SAAKOV, 2003]. An important advantage for obtaining carotenoids of fungal origin is the lack of seasonal dependence of biotechnological production, ecological purity of the obtained drugs, availability of raw materials [PYROG, IHNATOVA, 2009].

Light belongs to environmental factors and regulates morphogenetic processes in many types of fungi. The nature of the effect of light depends on its spectral characteristics and on the duration of the light [KAMADA et al., 2010]. Recently, the mechanisms of photoreception in fungi have been the subject of intensive research [FROEHLICH et al. 2005; DE FABO et al., 2008; KRITSKIY et al., 2010; CORROCHANO, GARRE, 2010; FULLER et al. 2015]. Mushrooms can absorb ultraviolet, blue, green, red and distant red light, using up to 11 photoreceptors and signaling cascades to control most of the genome and adapt to environmental conditions [YU, FISCHER, 2019]. Thus, in the agaricomicetes Coprinus cinereus (Schaeff.) Gray, Pleurotus ostreatus (Jacq.) P. Kumm. and Lentinula edodes (Berk.) Pegler genes encoding the receptors, responsible for the perception of blue light, were found. The study of the fungal genome revealed the photoreceptor genes encoding proteins that are sensitive to red light [KAMADA et al., 2010]. Green light is perceived by opsin systems based on retinal, whose biological functions still need to be clarified [YU, FISCHER, 2019]. The positive influence of irradiation on P. ostreatus fungus yields was investigated, and it was also found that laser irradiation at doses of 45-230 mJ/cm² stimulates sprout growth and mycelium growth in Hericium erinanceus. The known influence of low intensity light on linear growth and biomass accumulation by different types of macromycetes (Agaricus bisporus, Inonotus obliguus, Ganoderma lucidium, Hericium erinanceus, L. ededes) [Poyedinok et al., 2013]. It is known that the dependence of photoinduced stimulation of fungal mycelium growth on the concentration of the carbon source in the nutrient medium. In addition, it was found that irradiation leads to changes in the trophism of fungi, which is expressed in an increase in the efficiency of consumption of a carbon source in environments with low glucose content [POYEDINOK, 2015]. However, literature data on the influence of LED laser systems on the parameters of fungal growth when cultivated on a medium with different concentrations of carbon sourceare limited, so this issue requires further study. In view of the above, the aim of our article was to determine the amount of carotenoids in *L. sulphureus* mycelium under the influence of laser irradiation and to study the carotenoid accumulation in mycelium under the influence of irradiation at low glucose concentrations.

Materials and methods

Research were conducted at the Department of Botany and Ecology of Vasyl' Stus Donetsk National University. Three strains from the collection of agaricomycete cultures of the Department of Botany and Ecology of Vasyl' Stus Donetsk National University belonging to the Basidiomycota division were used for the research.

To obtain an inoculum the mycelium of strains Ls-17, Ls-16, Ls-18 of the fungus *L. sulphureus* was cultured for 7 days on agar potato-glucose medium in standard Petri dishes (9 cm in diameter).

A device designed by the staff of the Department of Botany and Ecology of Vasyl Stus Donetsk National University was used for laser irradiation of mycelium. The device consists of an 8–sided mirror prism, receives a beam of LED lasers: 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 emission of a green spectrum with a wavelength of 532 nm (laser manufacturer BOB LASER Co., China) and reflects it on a conveyor belt on which a Petri dish with mycelium is placed. The power of each laser is 100 mW. The device has 2 electric motors, which are responsible for the movement of the mirror prism and the conveyor belt. The device is controlled by a control panel, which is equipped with buttons to adjust the exposure time and select the desired laser with the appropriate wavelength of light. The mycelium was irradiated as follows: a Petri dish with mycelium moves along the conveyor belt and passes under a beam of light with a set wavelength: 635 nm or 405 nm or 532 nm, obtaining the necessary radiation energy (51.1 mJ/cm²), depending on the purpose of our study. Mycelium irradiation in our studies lasted 10 seconds. Mycelium irradiation was conducted in the following embodiments (table 1).

The irradiation of the mycelium of the studied species of macromycetes

Table 1.

Irradiation option	Irradiation duration, sec			Irradiation energy,
	Red light (wavelength 635 nm)	Blue light (wavelength 405 nm)	Green light (wavelength 532 nm)	mJ/cm²
	(wavelength 633 mm)	(wavelength 403 mm)	(wavelength 332 mm)	
1 (контроль)	0	0	0	0
2	10	0	0	51,1
3	0	10	0	51,1
4	0	0	10	51,1

Inoculation of Erlenmeyer flasks was performed under sterile conditions using a sterile steel tube 5 mm diameter. For inoculation one of Erlenmeyer flask with nutrient medium used five mycelial disks with a diameter of 5 mm. An unirradiated culture was used to inoculate control Erlenmeyer flasks.

To study the total carotenoid content of mycelium of strains L.s.-17, L.s.-16, L.s.-18 of the fungus *L. sulphureus* was cultured by stationary culture in Erlenmeyer flasks on glucosepeptone nutrient medium of the following composition (g/dm³) [BISKO et al., 1983]: different concentrations of glucose (10, 8, 6, 4), peptone - 3.0; KH₂PO4 - 0,6; K₂HPO4 - 0.4; MgSO₄·7 H2O - 0.5; CaCl₂ - 0.05; ZnSO₄·7 H₂O - 0.001, distilled water - 1 dm³. The volume of Erlenmeyer flask was 0.25 dm³, the volume of nutrient medium was 0.05 dm³. Duration of

cultivation - 12 days. Cultivation was carried out at a temperature of 25 \pm 2 °C in a thermostat.

The accumulation of biomass in all experiments was determined by the weight method, drying the mycelium to a constant mass at a temperature of 105° C [Dudka et al., 1982], (g absolute dry biomass /dm³). To determine the total carotenoid content, the mycelium was homogenized by grinding in a sterile mortar and extracted with ethyl alcohol (90%) in a ratio of 1:5 for 10 min at temperatures of 60 °C. The mixture was centrifuged for 10 minutes at RCF 450 (Laboratory medical centrifuge OPn-8), the supernatant was drained and used to determine the amount of carotenoids. Determination of the amount of carotenoids was performed in mycelium – per unit mass, g spectrophotometric (Granum 722, China) method and calculated by the modified Wettstein formula for the determination of carotenoids in fungi [WETTSTEIN, 1957].

$$C = 4.69 \times D440.5$$

where D440,5 – the optical density of the solution at wavelengths $\lambda = 440,5$ HM; Then calculated the pigment content in the test material in terms of wet weight [MUSIENKO et al., 2001].

$$A = \frac{C \cdot V}{n \cdot 1000}$$

A – the content of pigments in the test sample, mg/g biomass; C – the concentration of pigment found by the Wetstein formula, mg/dm³; V – volume of extract, cm³; n – sample of the prototype, g; 1000 – coefficient for calculating the concentration of pigments per 1 cm³. All experiments were performed in triplicate. To determine the probability of exposure to laser irradiation, the analysis of variance was used. Comparisons of average values were carried out by the method of Dunnett. Processing was carried out using a package of statistical programs created at the Department of Plant Physiology of the Vasyl' Stus Donetsk National University [PRYSEDSKYY, 2005].

Results and discussion

A study of the biomass accumulation in L. sulphureus mycelium due to irradiation at different glucose concentrations showed that for L. sulphureus macromycetes is most effective the use of glucose-peptone medium with a glucose concentration of 10 and 8 g/dm³ in combination with irradiation of mycelium with green light at a wavelength of 532 nm (irradiation energy 51.1 mJ/cm²). Under the influence irradiation of mycelium with green light, the best response was observed for the Ls-18 strain – the biomass in mycelium increased from 86.7 to 93.6% in accordance with the control. For strains Ls-17 and Ls-16, the biomass increased by 56.8-71,3% and by 57.5-60.3%, respectively. Laser irradiation of mycelium with blue light at a wavelength of 405 nm (irradiation energy 51.1 mJ/cm²) increased the number of biomass for strain Ls-18 by 86.7-93.6%, and for strains Ls-17 and Ls-16 by 56.8–58.1% and 57.5–60.3%, respectively. Irradiation with red light at a wavelength of 635 nm (irradiation energy 51.1 mJ/cm²) contributed to an increase in the number of biomass for all studied strains of the fungus L. sulphureus from 12.7% to 31.7%. Using a glucose-peptone medium with glucose concentrations of 6 and 4 g/dm³ in combination with laser irradiation of mycelium with red (wavelength 635 nm), blue (wavelength 405 nm) and green (wavelength 532 nm) light with an emission energy of 51.1 mJ/cm² no increased in the biomass of the mycelium (Fig. 1).

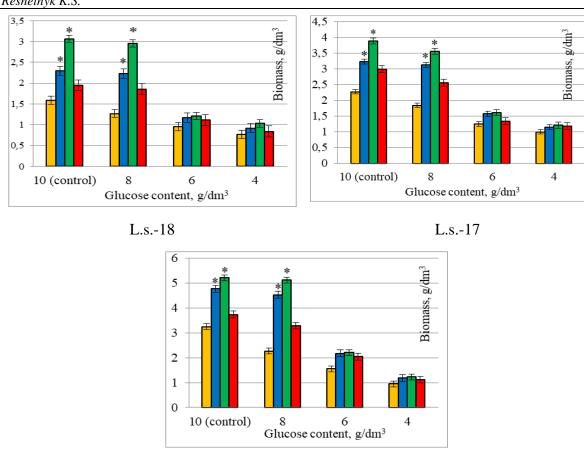


Fig. 1. Accumulation of absolute dry biomass (g/dm³) in the mycelium of Laetiporus sulphureus strain on glucose-peptone medium at different glucose content 12 day of cultivation. — without irradiation; 405 nm; − 635 nm; − 532 nm. Note. * – the difference is statistically significant compared to the control variant (P < 0.05).

L.s.-16

Since there is no information in the literature on the study of light exposure for this species of fungus, respectively, we first found that laser irradiation of the mycelium affects the content of carotenoid pigments of strains of the fungus L. sulphureus. A study of the carotenoid accumulation in L. sulphureus mycelium due to irradiation at different glucose concentrations showed that for L. sulphureus macromycetes (strain L.s.-17) is most effective the use of glucose-peptone medium with a glucose concentration of 10 and 8 g/dm³ in combination with irradiation of mycelium with green light at a wavelength of 532 nm (irradiation energy 51.1 mJ/cm²). Under these conditions, the carotenoid content significantly increased from 62.3 to 63.5% according to the control. Laser irradiation with blue light with a wavelength of 405 nm (irradiation energy 51.1 mJ/cm²) significantly increased the carotenoid content of mycelium from 30.6 to 32%. Irradiation with red light with a wavelength of 635 nm (irradiation energy 51.1 mJ/cm²) increased the content of carotenoids from 5.1 to 8.2%, respectively. Use of glucose-peptone medium with concentrations of 6 and 4 g/dm³ in combination with laser irradiation of green (wavelength 532 nm) light with irradiation energy of 51.1 mJ/cm², respectively, increased the content of carotenoids in the mycelium in insignificant values. Use of glucose-peptone medium with concentrations of 6 and 4 g/dm³ in combination with laser irradiation of red mycelium (wavelength 635nm), blue (wavelength 405 nm) light with irradiation energy of 51.1 mJ/cm², no increased the content of carotenoids in the mycelium (Fig. 2).

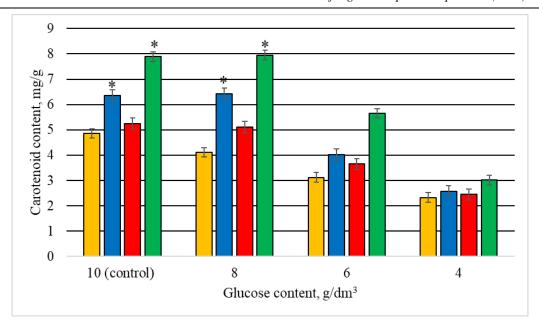


Fig. 2. The carotenoid accumulation (mg/g wet biomass) in the mycelium of L.s.-17 *Laetiporus sulphureus* strain on glucose-peptone medium at different glucose content. 12 day of cultivation. \square – without irradiation; \square – 405 nm; \square – 635 nm; \square – 532 nm. Note. * – the difference is statistically significant compared to the control variant (P <0.05).

The results of our studies for the strain L.s.-16 *L. sulphureus* show that it is advisable to use glucose-peptone medium with a glucose concentration of 10 and 8 g/dm³ in combination with irradiation of the mycelium with green light wavelength 532 nm (irradiation energy 51.1 mJ/cm²).

Under these conditions, the carotenoid content significantly increased from 37.1 to 61.9 % according to the control. Laser irradiation with blue light with a wavelength of 405 nm (irradiation energy 51.1 mJ/cm²) significantly increased the content of mycelial carotenoids from 15 to 40.5%. Irradiation with red light with a wavelength of 635 nm (irradiation energy 51.1mJ/cm²) increased the carotenoid content from by 28.9%. Use of a glucose-peptone medium with glucose concentrations of 6 and 4 g/dm³ in combination with laser irradiation of mycelium with red (wavelength 635 nm), blue (wavelength 405 nm) and green (wavelength 532 nm) light with an emission energy of 51.1 mJ/cm² no increased in the content of carotenoids in the mycelium (Fig. 3).

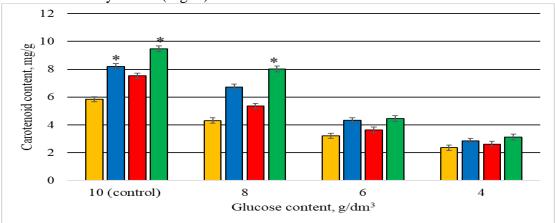


Fig. 3. The carotenoid accumulation (mg/g wet biomass) in the mycelium of strain L.s.-16 *Laetiporus sulphureus* on glucose-peptone medium at different glucose content. 12 day of cultivation. \square – without irradiation; \square – 405 nm; \square – 635 nm; \square – 532 nm. Note. * – the difference is statistically significant compared to the control variant (P <0.05).

The results of our studies for the *L. sulphureus* strain L.s.-18 show that, to increase the carotenoid content, it is advisable to use a glucose-peptone medium with a glucose concentration of also 10 and 8 g/dm³ in combination with green light irradiation waves 532 nm (irradiation energy 51.1 mJ/cm²). Under these conditions, the carotenoid content significantly increased from 44.8 to 66.1% according to the control. Laser irradiation with blue light with a wavelength of 405 nm (irradiation energy 51.1 mJ/cm²) significantly increased the content of mycelial carotenoids from 18.1 to 46.7%. Irradiation of the mycelium with red light with a wavelength of 635 nm (irradiation energy 51.1 mJ/cm²) increased the carotenoid content by 16.8%. Using a glucose-peptone medium with glucose concentrations of 6 and 4 g/dm³ in combination with laser irradiation of mycelium with red (wavelength 635 nm), blue (wavelength 405 nm) and green (wavelength 532 nm) light with an emission energy of 51.1 mJ/cm² no increased in the content of carotenoids in the mycelium (Fig. 4).

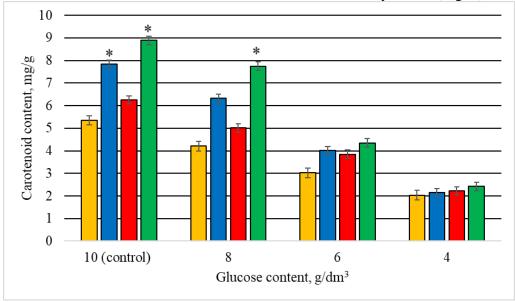


Fig. 4. The carotenoid accumulation (mg/g wet biomass) in the mycelium of strain L.s.-18 *Laetiporus* sulphureus on glucose-peptone medium at different glucose content. 12 day of cultivation. \Box – without irradiation; \Box – 405 nm; \Box – - 635 nm; \Box – 532 nm. Note. * – the difference is statistically significant compared to the control variant (P <0.05).

Analysis of the results of our studies for the fungus L. sulphureus shows that to increase the content of carotenoids it is advisable to use glucose-peptone medium with a glucose concentration of 10 and 8 g/dm³ in combination with mycelium irradiation with green light wavelength 532 nm (irradiation energy 51.1 mJ/cm²). Under these conditions, the statistically significant increase in the content of carotenoids in the mycelium was found from 37.1% to 66.1%. From literary sources it is known that L. sulphureus macromycetes are capable of synthesizing carotenoids, mainly under the influence of light [ZHDANOVA et al., 1982]. Because carotenoids are secondary metabolites, it is possible to regulate their synthesis by changing the conditions of cultivation of producer strains, including the composition of nutrient media. It is known that to increase the accumulation of carotenoids in the mycelium of L. sulphureus, it is advisable to introduce into the standard glucose-peptone medium peptone in concentration of 5 g/dm³, and also proline or valine [VELYGODSKA et al., 2014]. It is also proven that the source and amount of carbon can dramatically change the biosynthetic function of fungal organisms [PIROG, 2010]. The results of our studies show the increase in carotenoid content at low glucose concentration (8 g/dm³ instead of 10 g/dm³) in combination with irradiation of the mycelium with green light with a wavelength of 532 nm (irradiation energy 51.1 mJ/cm²) is a reaction fungal organism to stressful conditions that occur due to a decrease in the amount of carbon in the nutrient medium. As a result, there is a rapid adaptation of the fungal organism to negative changes in environmental conditions.

Conclusions

Thus, analyzing our research results, we can conclude that is most effective the use of glucose-peptone medium with a glucose concentration of 10 g/dm³ in combination with irradiation of mycelium with green light at a wavelength of 532 nm (irradiation energy 51.1 mJ/cm²). Under the action of this irradiation regime for strain L.s.-18 the content of carotenoids in the mycelium increased by 66.1% according to the control. Laser irradiation of mycelium with blue light with a wavelength of 405 nm (irradiation energy 51.1 mJ/cm²) increased the content of carotenoids for strain L.s.-18 by 46.7%. Irradiation with red light with a wavelength of 635 nm (irradiation energy 51.1 mJ/cm²) contributed to an increase in the content of carotenoids for strain L.s.-16 of the fungus L. sulphureus by 28.9%. It was found that the use of glucose-peptone medium with a glucose concentration of 8 g/dm³ in combination with irradiation of the mycelium with green light with a wavelength of 532 nm (irradiation energy 51.1 mJ/cm²) was less effective. Under these conditions, the content of carotenoids in the mycelium increased for strain L.s.-17 by 62.3%. Laser irradiation of mycelium with blue light with a wavelength of 405 nm (irradiation energy 51.1 mJ/cm²) increased the content of carotenoids for strain L.s.-17 by 30.6% respectively. Irradiation with red light with a wavelength of 635 nm (irradiation energy 51.1 mJ/cm²) contributed to an increase in the content of carotenoids for strain L.s.-18 of the fungus L. sulphureus by 16.8% respectively. For strain L.s.-16 the number of carotenoids in the mycelium no increase. The use glucose-peptone medium with glucose concentrations of 6 and 4 g/dm³ in combination with laser irradiation of mycelium with red (wavelength 635 nm), blue (wavelength 405 nm) and green (wavelength 532 nm) light with irradiation energy 51.1 mJ/cm² was no increase in the content of carotenoids in the mycelium.

References

- BECKER Z.E. (1988). *Physiology and biochemistry of fungi*. Moscow: Publishing house of Moscow University, 230 p. (in Russian)
- BISKO N.A., BUKHALO A.S., WASSER S.P., DUDKA I.A., KULESH M.D., SOLOMKO E.F., SHEVCHENKO S.V. (1983). *Higher edible basidiomycetes in surface and deep culture*. K.: Science. Dumka, 312 p. (in Russian)
- BRITTON G. (1986). Biochemistry of natural pigments. Per. from English Moscow: World, 442 p. (in Russian)
- BUTSENKO L.M., PENCHUK YU.M., PYROG T.P. (2010). *Technologies of microbial synthesis of drugs*. Kyiv: NUHT, 323 p. (in Ukrainian)
- CORROCHANO L.M., GARRE V. (2010). Photobiology in the Zygomycota: multiple photoreceptor genes for complex responses to light. *Fungal Genet. Biol.*, **47**: 893–899.
- DE FABO E.C., FRIEDL M.A., SCHMOLL M., KUBICEK C.P., DRUZHININA I.S. (2008). Photostimulation of Hypocrea atroviridis growth occurs due to a crosstalk of carbon metabolism, blue light receptors and response to oxidative stress. *Microbiology*, **154**: 1229–1241.
- DUDKA I.A., WASSER S.P., ELLANSKAYA I.A. (1982). *Methods of experimental mycology*. Reference. Kyiv: Scientific opinion, 561 p. (in Russian)
- ELDAHSHAN O.A., SINGAB A.N. (2013). Carotenoids. J. Pharmacogn. Phytochem. 2(1): 225-234.
- FEDOTOV O.V. (2007). Wood-destroying fungi as bio-sources of ferments for medicinal and nutritional purposes. Plant and microbial enzymes: Isolation, characterization and biotechnology applications. *Myza, Tbilisi*, 125–131.
- FROEHLICH A.C., NOH B., VIERSTRA R.D., LOROS J., DUNLAP J.C. (2005). Genetic and molecular analysis of phytochromes from the filamentous fungus Neurospora crassa. *Eukaryot. Cell.*, **4**: 2140–2152.
- FULLER K.K., LOROS J.J., DUNLAP J.C. (2015). Fungal photobiology: visible light as a signal for stress, space and time. *Curr Genet.*, **61**: 275–288.
- GESSLER N.N., SOKOLOV A.V., BYKHOVSKY V.YA., BELOZERSKAYA T.A. (2002). Superoxide dismutase and catalase activity in Blakeslea trispora and Neurospora crassa karatinoid-synthesizing fungi under conditions of oxidative stress. *Applied Biochemistry and Microbiology*, **8** (3): 237–242. (in Russian)
- GESSLER N.N., OKOLOV A.B., BELOZERSKAYA T.A. (2003). Participation of β-carotene in the antioxidant protection of the fungal cell. *Applied Biochem. and Microbiol.*, **39** (4): 427–429. (in Russian)

- GESSLER N.N., LEONOVICH O.A., RABINOVICH M.YA. (2006). A comparative study of the components of antioxidant defense during the growth of wild-type mycelium Neurospora crassa and mutants white color 1 and white color 2. *Applied Biochemistry and Microbiology.*, **42** (3): 332–337. (in Russian)
- GOODWIN T.W. (1980). The Biochemistry of carotenoids. Plants. Chapman & Hall, London, 1: 315 p.
- KAMADA T., SANO H., NAKAZAWA T., NAKAHORI K. (2010). Regulation of fruiting body photomorphogenesis in Coprinopsis cinerea. *Fungal Genet Biol.*, **47**(11): 917–921.
- KARNAUKHOV V.I. (1986). Biological functions of carotenoids. Moscow: Nauka, 223 p. (in Russian)
- KRITSKIY M.S, TELEGINA T.A, VECHTOMOVA Y.L, KOLESNIKOVA M.P, LYUDNIKOVA T.A, GOLUB O.A. (2010). Photoexcited molecules of flavin and pterin coenzymes in evolution. *Biochemistry*., **75**(10): 1348–1366.
- MUSIENKO M.M., PARSHIKOVA T.V., SLAVNYI P.S. (2001). Spectrophotometric methods in the practice of physiology, biochemistry and plant ecology. Kyiv: Fitosotsiocenter, 200 p. (in Russian)
- PIROG T.P., BUTSENKO L.M., PENCHUK Y.M. (2010). *Technologies of microbial synthesis of drugs*: Textbook. way. Kyiv: NUHT, 323 p. (in Ukrainian)
- POYEDYNOK N.L. (2013). The Use of artificial light in mushroom cultivation biotechnologies. *Biotechnology Acta*, **6**(6): 58–70.
- POYEDYNOK N.L. (2015). Biotechnological basis of intensification cultivation of edible and medicinal macromycetes with low light intensity. DSc thesis. Kyiv: Institute of Food Biotechnology and genomics. (in Russian)
- PYROG T.P., IHNATOVA O.A. (2009). General biotechnology [Electronic resource]: textbook. Kyiv: NUHT, 336 p. (in Ukrainian)
- PRYSEDSKYY Y.G. (2005). Software package for statistical processing of the results of the biological experiments. Donetsk, 84 p. (in Ukrainian)
- RIBEIRO B., GUEDES DE PINHO P., ANDRADE P.B., OLIVEIRA C., CÉSAR A., FERREIRA S., BAPTISTA P., VALENTÃO DO P. (2011). Bioactive Carotenoids Contribute to the Color of Edible Mushrooms? *The Open Chemical and Biomedical Methods Journal*, **4**: 14–18.
- SAAKOV V.S. (2003). Alternative pathways of carotenoid biosynthesis in Procaryota and Eucaryota. *Dokl. Academy of Sciences of Russia.*, **392**(6): 825–831.
- VELYGODSKA A.K., FEDOTOV O.V., PETREEVA A.S. (2014). Effect of nitrogen nutrition sources on carotenoids synthesis for some basidiomycetes strains. *Biological Bulletin of Bogdan Chmelnitskiy Melitopol State Pedagogical University*, **4**(1): 22–34 (in Ukrainian).
- VELYGODSKA A.K., FEDOTOV O.V. (2016). Obtaining and analysis of carotenoid preparations of some strains of xylotrophic basidiomycetes. *Bulletin of Dnipropetrovsk University*. *Biology*, *ecology*, **4** (2): 290–294. (in Ukrainian)
- WETTSTEIN D. (1957). Chlorophyll-letale und der submikroskopishe Formweschsel der Plastiden. *Exp Cell Res.*, **12**(3): 427–506.
- YUZ., FISCHER R. (2019). Light sensing and responses in fungi. Nature Reviews Microbiology, 17(1): 25–36.
- ZHDANOVA N.N., VASILEVSKAYA A.I. (1982). Extreme ecology of mushrooms in nature and experiment. Kiev: Naukova Dumka, 168 p. (in Russian)