Influence of low-intensity light on the biosynthetic activity of the edible medicinal mushroom *Hericium erinaceus* (Bull.: Fr.) Pers. *in vitro*

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Abstract: Understanding the impact of light on the physiology and metabolism of edible and medicinal mushrooms can step up the technologies aimed at obtaining bioactive compounds. The article presents data on the influence of low-intensity quasimonochromatic light on the biosynthetic activity of *Hericium erinaceus* edible medicinal mushrooms *in vitro*. An artificial lighting setup based on light-emitting diode (LED) arrays with wavelengths \(\lambda=470\ nm\) (blue), \(\lambda=530\ nm\) (green), and \(\lambda=650\ nm\) (red) was used. An argon gas laser served as a coherent visible light source at a wavelength of 488 nm. The mycelium of *H. erinaceus* irradiation by low-intensity light at wavelengths of 488 nm, 470 nm, and 650 nm reduced the lag phase and increased the culture’s growth rate. The highest biomass yield (12.1 g/L) on the 12\(^{th}\) day of cultivation was achieved with light irradiation at a wavelength of 488 nm. Irradiation of the mycelium of *H. erinaceus* in all used wavelength ranges led to an increase in the synthesis of polysaccharides and unsaturated fatty acids. The modes of irradiation with laser light \(\lambda=488\ nm\) and LED \(\lambda=470\ nm\) were the most effective.

Keywords: LED, mycelial mass, polysaccharides, fatty acids

Abbreviations: light-emitting diode (LED); mushroom culture collection of the M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine (IBK); extracellular polysaccharide (EPS); intracellular polysaccharides (IPS); monounsaturated fatty acids (MUFA); polyunsaturated fatty acids (PUFA); saturated fatty acids (SFA)

INTRODUCTION

Edible medicinal mushrooms offer significant biotechnological potential for various industrial applications [1,2]. Given the new trend towards a healthy lifestyle, there has been a significant increase in demand for natural, environmentally friendly organic food products and pharmaceuticals. For thousands of years, human society has been using edible macromycetes as food because they are an excellent source of protein and essential amino acids, low fat content, and cholesterol-free [3-5]. Furthermore, mushrooms are used in biotechnology because they synthesize valuable metabolites with a proven pharmacological potential [2,6]. One such valuable cultivated species is *H. erinaceus*, also known as Lion’s Mane. This mushroom species has been used in traditional Chinese medicine for over 2,000 years because of its medicinal properties. The fruiting bodies and mycelial mass of *H. erinaceus* are rich sources of proteins, amino acids, minerals, vitamins, and bioactive compounds [7,8]. This species can be considered a beneficial food product for patients with dietary restrictions and vegans who require alternative sources of essential nutrients [9].

In addition to its high nutritional value, the pharmacological importance of *H. erinaceus* has also been proven. The study of the mycochemical composition of...
the fruiting bodies and mycelial mass of *H. erinaceus* has led to the identification and characterization of 70 to 102 different bioactive compounds, according to various data sources [10,11]. In the past few decades, large amounts of metabolites such as polysaccharides, erinacines, erinacerins, erinaceolactones, glycoprotein, terpenoids, phenols, organic and amino acids, carbohydrates and derivatives, and unsaturated fatty acids have been isolated from the *H. erinaceus* fruiting body, and the health-promoting properties of the fruiting body have been extensively reviewed [12]. The secondary metabolites of *H. erinaceus* exhibit a wide range of biological activities, including immunomodulatory and antitumor properties and antiviral, antimicrobial, and anti-inflammatory activities [13-15]. It has been shown that two classes of terpenoid compounds, namely hericenones and erinacines, isolated from the fruiting bodies and cultivated mycelium of *H. erinaceus*, respectively, stimulate the synthesis of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF). NGF and BDNF are responsible for the regeneration of neurological disorders and may aid in preventing and treating neurodegenerative diseases [16-18].

Considering the nutritional value, unique chemical composition, and proven pharmacological activity of *H. erinaceus*, the intensification of its cultivation processes using light of various wavelengths based on LED can be of practical and scientific interest. Low-intensity artificial light based on LED is one of the environmentally friendly regulators of the life processes of fungi [19,20]. Light and its parameters (wavelength, intensity, duration) significantly influence the adaptation processes of fungi to the surrounding environment, including mycelium growth, its chemical composition, and antioxidant and antimicrobial activity [18, 21-24].

The light-dependent nature of biosynthesis has been established in fungi for certain groups of compounds, including carotenoids, carbohydrates, nucleotides, amino acids, and fatty acids [25,26]. Studies on the effects of light exposure on the vitality of fungi have mainly been conducted on microfungi [24,27], while there is a lack of data regarding the specifics of the influence of quasimonochromatic light from LED sources on the biosynthetic activity of edible and medicinal macromycetes in vitro. Our previous research investigated the impact of low-intensity laser irradiation on spore germination, growth processes, and fruiting body formation of *H. erinaceus* [28,29]. Understanding the physiology and assessing the ways of using LED to influence the metabolism of *H. erinaceus* can positively impact future research and provide knowledge for the intensification and regulation of cultivation processes for this valuable species of macromycete.

This work aimed to investigate the influence of low-intensity quasimonochromatic light on the biosynthetic activity of the edible medicinal mushroom *H. erinaceus* under submerged cultivation conditions.

**MATERIALS AND METHODS**

**Mushroom strain**

The subject of the research was a pure culture of the medicinal basidiomycete *H. erinaceus* IBK 977, which is preserved in the Mushroom Culture Collection at the M.G. Khododny Institute of Botany, National Academy of Sciences of Ukraine (acronym IBK) [30]. The taxonomic status of the *H. erinaceus* IBK 977 strain was confirmed at the species level. The obtained data are deposited in the NCBI database available at GenBank (https://www.ncbi.nlm.nih.gov/genbank/) with the accession number MF952886. Based on preliminary cultural and morphological studies, characteristic taxonomic features have been identified in the vegetative stage of the culture, which can be used to ensure the purity of the *H. erinaceus* IBK 977 culture at all stages of cultivation [31].

**The impact of artificial low-intensity irradiation using LED on biosynthetic activity**

The following scheme was employed to determine the influence of the light factor on the biosynthetic activity and fatty acid composition of the mycelium of the *H. erinaceus* IBK 977 strain (and is summarized in Supplementary Fig. S1).

The mother culture was transferred to Petri dishes (d=90 mm) containing glucose-peptone-yeast agar (GPYA) composition: 2.5% (w/v) glucose, 0.3% (w/v) peptone, 0.2% (w/v) yeast extract, 0.1% (w/v) KH₂PO₄, 0.1% (w/v) K₂HPO₄, 0.025% (w/v) MgSO₄·7H₂O, 2.0% (w/v) agar-agar. The Petri dishes with the cultures were
incubated at 26°C in the dark. After complete coloniza-
tion of the agar surface with mycelium, the vegetative
mycelium and the agar medium were homogenized
using Homogenizer type-302 (Warsaw, Poland) under
sterile conditions. The obtained homogenate of myce-
lial mass in a volume of 10% of the total volume was
inoculated into 0.5 L Erlenmeyer flasks with 100 mL
of a liquid glucose-peptide-yeast nutrient medium
(GPY), as described above, without agar-agar, pH 5.5.
Inoculation was performed using the physiologically
active mycelium according to the methodology devel-
oped for mycelial fungi [32]. Subsequently, cultivation
was carried out on an orbital shaker (120 rpm) at
26°C. The liquid-to-air phase ratio in the flasks was
maintained at 1:5.

An artificial lighting system based on LED matrices
was used to determine the influence of quasimono-
chromatic light as a factor on the biosynthetic activ-
ity of *H. erinaceus* IBK 977 mycelium. The lighting
system was developed at the Institute of Physics of the
National Academy of Sciences of Ukraine. The setup
for irradiating the mycelial cultures included a light-
emitting panel with LED matrices, a cooling system
with light-reflecting surfaces, LED power supplies,
and control units. The LED matrix was composed
of 21 high-power AlGaInN-based LEDs of the YSH-
FRGBB-IA type, manufactured by China Young Sun
Led Technology Ltd. Each LED module had three light-
emitting microchips, emitting light at wavelengths of
λ=470 nm (blue), λ=530 nm (green), and λ=650 nm
(red). A light-emitting panel was used as a source of
spectral-tailored light and served as the main com-
ponent of the lighting system. The panel dimensions
are 350×700 mm, which allows for the placement of
flasks or Petri dishes with the material samples to be
irradiated with light of different wavelengths as needed.
It is equipped with 21 LEDs with independent power
control for each of the three spectral channels (blue,
green, and red), a ballast resistor block, and fans for
the air-cooling system of the LED. The intensity of the
light was adjusted using an external control unit. Each
microchip had an electrical power of 1 watt, and the
intensity of the emitted light could be adjusted from
zero to the maximum value independently for each
spectral range, i.e., separately for blue, green, and red
light, by controlling the current strength through the
diodes. The power supply units were located on the
side panel of the lighting system.

The 0.5-L glass flasks containing the inoculated
material, the thickness of the medium layer with sub-
merged mycelium (1.0 cm), were placed on a glass
shelf underneath the light-emitting panel. The cal-
culated parameters of the lighting system are given
in Supplementary Table S1.

A gas argon laser was used as a coherent visible
light source at a wavelength of 488 nm. The power
density of the laser emission was monitored using an
optical digital power and energy meter, PM-100D from
Thorlabs Inc., with a standard photodiode power sensor,
S120C, operating in the range of 400-1100 nm. The light
energy dose (the amount of light energy incident on a
unit area) is determined by the product of the power
density and the exposure time. In all experimental
conditions, equal light energy doses were selected for
the effects on the vegetative mycelium of mushrooms,
ensuring that the energy density on the sample surface
was consistent. In our experiments, this value was
set to 240 mJ/cm². The irradiation was conducted in
continuous mode. In studying the light's influence on
growth and biosynthetic activity, both non-irradiated
(control) and irradiated mycelium were used.

When selecting the same energy conditions for
irradiating the samples with light, we proceeded from
the assumption that in the absence of established and
universally accepted mechanisms of low-intensity
radiation's effects on mycelium, it would be justified
to focus on determining qualitative differences in the
impact of equal doses of radiation energy with differ-
ent spectral composition and temporal characteristics
on them.

After irradiation, the *H. erinaceus* IBK 977 myceli-
um was inoculated in 10% volume into 0.5 L Erlenmeyer
flasks containing 100 mL of liquid GPY medium for
further cultivation under submerged culture conditions.

The mycelium biomass was separated from the
culture broth obtained under submerged cultivation
conditions by filtration through Whatman No.1 filter
paper. The mycelium was then dried to a constant
weight in a forced-air thermostat (model TSO-80
MICROmed, Shanghai Youding International Trade
Co., Ltd. (China)) at a temperature of 60°C. The dry
biomass was calculated in g/L considering the weight
of the inoculum. A mycelial mass (4.0 g) was taken for
the determination of the fatty acid profile.
Determination of fatty acid composition

The fatty acid composition of the mycelial mass samples was analyzed by gas chromatography of fatty acid methyl esters (ISO 5508:1990, IDT) using a Crystallux 4000M gas chromatograph. A chromatographic column DB-225 (l=30 m, d=0.32 mm and film thickness 0.25 μm) with a sorbent ((50%-cyanopropylphenyl)-dimethylpolysiloxane (Agilent, USA) was used.

Conditions of sample preparation

Sample preparation was based on the alkaline hydrolysis of triglycerides to free fatty acids followed by the esterification reaction of methyl esters of fatty acids. Fatty acid methyl esters were prepared according to ISO 5509:2000, IDT. The resulting solution of methyl esters of fatty acids in a volume of 1 μL was introduced into the chromatograph.

Chromatography conditions

The initial temperature of the column was 45ºC with exposure for 3 min and increased at a rate of 25ºC/min to 195ºC, to 205ºC at a rate of 3ºC/min, followed by an increase at a rate of 8ºC/min up to a temperature of 215ºC and hold for 1 min. The FID temperature of the detector was 270ºC, and the evaporator temperature was 250ºC. The pressure on the capillary column was 0.8 atmospheres, which creates a constant nitrogen carrier gas flow rate of 2.9 mL/min. Fatty acid methyl esters were identified by comparing the retention time of the peaks with the parameters of the peaks of the reference sample FAME Mix, № 18919-1AMP Supelco (USA) x (mixtures of 37 methyl esters of known composition).

Determination of intracellular polysaccharides

After filtration of the cultural liquid, the mycelium was washed with distilled water and dried to dry biomass (d.b.) at 60ºC. For IPS extraction, the whole mycelium obtained from one flask was dried at 60ºC, homogenized in a laboratory blender, then supplemented with distilled water (1:10 by weight), and boiled in a water bath (100ºC) for 18 h. Cytoplasmic contents were removed by multiple centrifugations (3,000 xg for 15 min) of the homogenized mycelium suspended in distilled water. The washing procedure was stopped only when the optical density of the supernatant at 280 nm did not exceed 0.1 32. The obtained extracts were concentrated two- or three-fold with a rotary evaporator (60ºC), treated with 96% ethanol (volume ratio 1:1) at a temperature of 4ºC, and allowed to stand until complete precipitation. The precipitate (IPS fractions) was separated by centrifugation and then dialyzed against distilled water for 3 days. The dialyzed IPS was precipitated with ethanol (volume ratio 1:2), washed with ethanol, ether, and acetone, and dried at 37ºC. The IPS content was calculated in % of absolute dry biomass. The IPS production (mg/L) was calculated as the total amount of IPS per L of medium consumed for mycelium cultivation over the entire cultivation period.

Determination of extracellular polysaccharides

The content of EPS in the culture liquid was determined by the weight method. For the determination of EPS, the obtained culture fluid after mycelia removal was concentrated in a vacuum evaporator three times from the initial volume, precipitated with 96% cooled ethanol in a ratio of 1:1, and placed in a refrigerator at 4ºC for 24 h. The precipitate was isolated from the supernatant by centrifugation at 8,000×g for 15 min. After separation, EPS was dried at 60ºC to constant weight. The yields of EPS were expressed as the g dry weight/L of the culture liquid [15].

Determination of the effectiveness of the light treatments

The impact of light irradiation on biosynthetic activity was assessed by monitoring changes in the accumulation of mycelial mass, polysaccharides, and the fatty acid profile of the mycelial mass. The results were calculated as percentages (%) relative to the control group (where the control group was considered 100%).

Statistical analysis

The study was performed in triplicate. The results of the quantitative content of fatty acids in the mycelium samples were considered the average value of three replicates. Statistical data processing was carried out using the methods of descriptive statistics, regression, and variance analysis using the Statistica 10.0 program.
The significance of the experimental data was assessed using variance analysis. Results with values of P≤0.05 were considered statistically reliable.

RESULTS

It was established that irradiation of inoculation mycelium *H. erinaceus* IBK 977 with low-intensity light during submerged cultivation leads to a reduction of the lag phase and an increase in the growth rate of the culture (Fig. 1). During growth of the mycelium, a reduction of the lag phase by 2 days was observed, compared to the control, when irradiated with light in the blue (LED λ=470 nm; laser λ=488 nm) and red (LED λ=650 nm) range wavelengths. According to the results, it was established that light treatment could significantly shorten the cultivation period of *H. erinaceus* IBK 977 when obtaining the mycelial mass. The mycelium of this strain was more sensitive to laser light λ=488 nm, the irradiation of which made it possible to obtain the highest biomass yield (12.1 g/L) on the 12th day of cultivation. The accumulation of mycelial mass using blue light λ=470 nm and red light λ=650 nm was 10.5 g/L and 10.1 g/L and did not differ statistically (Fig. 1). The use of green light λ=530 nm was the least effective of all the modes used, the accumulation of mycelial mass on the 14th day was 8.8 g/L.

**Effect of irradiation on the synthesis of polysaccharides**

Analysis of the formation of polysaccharides in all variants of the experiment showed that the accumulation of mycelial mass and polysaccharides correlate with each other (Table 1). Irradiation of the inoculum with LED in all used wavelength ranges caused an increase in the synthesis of both EPS and IPS. The most effective modes of irradiation were the laser light (λ=488 nm) and LED (λ=470 nm). Under these regimes, the amount of EPS accumulation increased by 47.6% and 42.8%, respectively. Irradiation with red (λ=650 nm) and green (λ=530 nm) light induced an increase in the synthesis of EPS in *H. erinaceus* IBK 977 by 33.3% and 19.0%, respectively. The same trend was observed when analyzing the amount of IPS in the mycelial mass.

The results obtained allow us to state that low-intensity light in the visible part of the spectrum can be used in the biotechnology of submerged cultivation of *H. erinaceus* IBK 977 not only as a growth stimulator but also as a stimulator of polysaccharide synthesis (Table 1).

A total of 24 fatty acids were identified in the experimental samples of the mycelial mass of *H. erinaceus* IBK 977, ten of them are SFA, and fourteen are MUFA and PUFA (Table 2). The main fatty acids in the composition of all studied samples were linoleic acid (C\(_{18:2\omega-6}\)), oleic acid (C\(_{18:1}\)), and palmitic acid (C\(_{16:0}\)) because their content was 19-49%. Comparative analysis of the lipid fraction of the mycelial mass of *H. erinaceus* IBK 977 made it possible to establish that changes in the fatty acid profile of the mycelium occurred during LED irradiation of different wavelengths. In the control sample (without irradiation), the content of SFA was the highest and amounted to 21.97%. During irradiation in all modes, the content of SFA decreased (Fig. 2). The smallest number of SFA was recorded when

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**Table 1.** The influence of low-intensity quasimonochromatic light on the biosynthesis of polysaccharides of *Hericium erinaceus* IBK 977.

<table>
<thead>
<tr>
<th>Radiation wavelength (nm)</th>
<th>Biomass, g/L</th>
<th>Polysaccharides</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EPS, g/L</td>
<td>% changes IPS, %</td>
<td>% changes</td>
</tr>
<tr>
<td>Control without light exposure</td>
<td>5.3±0.2</td>
<td>2.1±0.3</td>
<td>4.3±0.2</td>
</tr>
<tr>
<td>λ=650.0</td>
<td>9.9±0.2*</td>
<td>2.8±0.2*</td>
<td>33.3</td>
</tr>
<tr>
<td>λ=530.0</td>
<td>8.1±0.3*</td>
<td>2.5±0.2*</td>
<td>19.0</td>
</tr>
<tr>
<td>λ=488.0</td>
<td>12.1±0.3*</td>
<td>3.0±0.3*</td>
<td>47.6</td>
</tr>
<tr>
<td>λ=470.0</td>
<td>10.5±0.2*</td>
<td>3.1±0.2*</td>
<td>42.8</td>
</tr>
</tbody>
</table>

* – statistically significant differences relative to control are indicated (P ≤0.05). Data are presented as the mean±standard error for three replications.
exposed to red light – 15.62%. The main component of SFA in all variants of the experiment was palmitic acid; its amount varied from 14.59% at λ=650 nm irradiation to 20.05% at laser irradiation (λ=488 nm). Changes in the qualitative composition of the fatty acid profile of the mycelial mass occurred under light exposure. It should be noted the absence or insignificant amount of tridecanoic acid (C13:0), heneicosanoic acid (C21:0), behenic acid (C22:0), lignoceric acid (C24:0) in samples exposed to irradiation. It was ascertained that MUFA and PUFA comprise most of the total amount of fatty acids in the mycelial mass of *H. erinaceus*. Minor changes in the concentration of MUFA were observed in the samples during irradiation in different modes (Fig. 2).

The amount of PUFA prevailed in all the studied samples (Table 2). According to health recommendations, the PUFA/MUFA ratio, which indicates the nutritional quality of dietary lipids, should be >0.4. In our study, the highest value of the PUFA/MUFA ratio was 1.8 when exposed to red light, and the lowest was 1.3 when exposed to laser light (λ=488 nm). Three

<table>
<thead>
<tr>
<th>No.</th>
<th>Common Name of Fatty Acid</th>
<th>Irradiation modes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>1.</td>
<td>Tridecanoic acid (C13:0)</td>
<td>ND</td>
</tr>
<tr>
<td>2.</td>
<td>Myristic acid (C14:0)</td>
<td>0.2499</td>
</tr>
<tr>
<td>3.</td>
<td>Pentadecanoic acid (C15:0)</td>
<td>0.6312</td>
</tr>
<tr>
<td>5.</td>
<td>Margaric acid (C17:0)</td>
<td>0.4795</td>
</tr>
<tr>
<td>6.</td>
<td>Stearic acid (C18:0)</td>
<td>0.8820</td>
</tr>
<tr>
<td>7.</td>
<td>Arachidic acid (C20:0)</td>
<td>0.01625</td>
</tr>
<tr>
<td>8.</td>
<td>Heneicosanoic acid (C21:0)</td>
<td>0.4037</td>
</tr>
<tr>
<td>9.</td>
<td>Behenic acid (C22:0)</td>
<td>ND</td>
</tr>
<tr>
<td>10.</td>
<td>Lignoceric acid (C24:0)</td>
<td>0.7076</td>
</tr>
<tr>
<td>11.</td>
<td>Myristoleic acid (C14:1 ω-5)</td>
<td>0.3552</td>
</tr>
<tr>
<td>12.</td>
<td>Pentadecanoic acid (C15:1)</td>
<td>1.335</td>
</tr>
<tr>
<td>13.</td>
<td>Palmitoleic acid (C16:1 ω-7)</td>
<td>0.5335</td>
</tr>
<tr>
<td>14.</td>
<td>Heptadecenoic acid (C17:1)</td>
<td>0.5461</td>
</tr>
<tr>
<td>15.</td>
<td>Oleic acid (C18:1 ω-9)</td>
<td>27.75</td>
</tr>
<tr>
<td>16.</td>
<td>Gondoic acid (C20:1 ω-9)</td>
<td>ND</td>
</tr>
<tr>
<td>17.</td>
<td>Erucic acid (C22:1 ω-9)</td>
<td>0.03435</td>
</tr>
<tr>
<td>18.</td>
<td>Linoleic acid (C18:2 ω-6)</td>
<td>46.96</td>
</tr>
<tr>
<td>19.</td>
<td>Eicosadienoic acid (C20:2 ω-6)</td>
<td>ND</td>
</tr>
<tr>
<td>20.</td>
<td>Arachidonic acid (C20:4 ω-6)</td>
<td>ND</td>
</tr>
<tr>
<td>21.</td>
<td><em>EPA</em> (cis-5,8,11,14,17-eicosapentaenoic acid, C20:5 ω-3)</td>
<td>ND</td>
</tr>
<tr>
<td>22.</td>
<td><em>ETA</em> (cis-8,11,14-eicosaatrienoic acid, C20:3 ω-3)</td>
<td>ND</td>
</tr>
<tr>
<td>23.</td>
<td><em>ETA</em> (cis-11,14,17-eicosaatrienoic acid, C20:3 ω-3)</td>
<td>ND</td>
</tr>
<tr>
<td>24.</td>
<td><em>DHA</em> (cis-4,7,10,13,16,19-docosahexaenoic acid, C22:6 ω-3)</td>
<td>ND</td>
</tr>
</tbody>
</table>

ΣMUFA+ ΣPUFA 77.51 78.27 82.14 83.92 78.24
ΣPUFA/ΣMUFA 1.54 1.39 1.51 1.82 1.13
ΣMUFA+ ΣPUFA/ ΣSFA 4.62 5.24 3.61

ND – not determined. The results of the content of fatty acid composition were considered the average value between three repetitions.
species of ω-9 MUFA (C₁₈:₁ ω-9, C₂₀:₁ ω-9, C₂₂:₁ ω-9), three of ω-3 MUFA (C₂₀:₅ ω-3, C₂₀:₃ ω-3, C₂₂:₃ ω-3), four of ω-6 MUFA (C₁₈:₂ ω-6, C₂₀:₃ ω-6, C₂₀:₂ ω-6, C₂₀:₄ ω-6), which belong to the group of essential fatty acids, were identified in the *H. erinaceus* mycelium samples. The synthesis of the listed fatty acids took place mainly under the irradiation of λ= 530 nm and λ= 650 nm. The greatest effect of LED irradiation on the synthesis of PUFA was recorded when exposed to red light (λ=650 nm). The minimum content of linoleic acid (36.72%) was recorded when exposed to laser light (λ=488 nm). It should be noted that in the control sample (without irradiation), only linoleic acid (C₁₈:₂ ω-6) is present in the PUFA, while irradiation with all other modes used contributed to an increase in newly formed fatty acids, i.e., an improvement in the qualitative composition of PUFA. Upon irradiation with green (λ=530 nm) and red light (λ=650 nm), six PUFA absent in the control were identified in the mycelial mass. When analyzing the data on the concentration of fatty acids with a chain length of C₁₈, oscillations in the levels of the content of saturated (C₁₈:₀) and unsaturated compounds (C₁₈:₁ ω₉; C₁₈:₁ ω₇; C₁₈:₂ ω₆) under different light regimes were observed. All the above fluctuations in the SFA/MUFA/PUFA content can be considered as the result of enzymatic reactions of the inducible type. In response to the influence of such an environmental factor as irradiation, the cell synthesizes inducible enzymes, in this case desaturase, which catalyzes the transformation of a single bond between carbon atoms in acyl chains (C–C) into double bonds (C–C).

DISCUSSION

Light is a key signaling element for growth and metabolic pathways in fungi [33]. Fungi can perceive near ultraviolet, blue, green, red, and far-red light, using up to 11 photoreceptors and signaling cascades to control most of the genome and thereby adapt to environmental conditions [34]. The blue light photoreceptor functions directly as a transcriptional regulator in the nucleus, while red and far-red light-sensitive phytochrome induces a signaling pathway for signal transduction from the cytoplasm to the nucleus. Greenlight can be sensed by retinal-binding proteins known as opsins, but the signaling mechanisms are poorly understood. When studying photobiology in fungi, the model objects were mainly micromycetes. However, recent studies have been carried out to study the effect of light on various aspects of the development and metabolism of edible and medicinal mushrooms, such as the accumulation of secondary metabolites, formation of fruiting bodies [35–38], the role of blue light receptors in fruiting and morphogenesis [39].

As for publications on the study of the spectral sensitivity of light exposure to the biosynthetic activity of macrofungi under submerged cultivation conditions, they are few. The intensification of obtaining mycelial mass and polysaccharides under submerged cultivation of edible and medicinal mushrooms, including *H. erinaceus*, was carried out mainly by optimizing the composition of nutrient media [32].

Previously, we carried out studies on the effect of laser radiation at doses of 45–230 mJ/cm² on the process of spore germination in *H. erinaceus* [28]. Using low-intensity laser radiation as a stimulator made it possible to triple the growth rate of monospore cultures. The effect of low-intensity light obtained from various sources on the linear growth and accumulation of biomass of vegetative mycelium by various types of macrofungi (*Lentinula edodes*, *Pleurotus ostreatus*, *H. erinaceus*, *Ganoderma lucidum*, *Inonotus obliquus*, *Agaricus bisporus*) was studied [23,40]. It was found that irradiation of the inoculum with blue and red light leads to the activation and increase in the growth rate of cultures, the reduction of the fermentation time under submerged cultivation and the period preceding fruiting, as well as the fruiting time in solid-phase fermentation. At the same time, there
is a significant increase in the yield of fruiting bodies and an improvement in their quality.

Our study was focused on establishing the effect of LEDs of different wavelengths on the biosynthetic activity of the \textit{H. erinaceus} IBK 977 strain under submerged cultivation conditions. When choosing the irradiation mode, we were guided by the data obtained by other researchers and the results of our previous studies [35-38,40,41]. When evaluating the biochemical effects of light, we considered the energy of the light quantum, the intensity of the light flux (the number of light quanta per unit area per unit time), the dose, and the spectral composition of light. From this point of view, LED can provide, even at low and medium intensity, a spectral density (energy per single frequency interval) that is not available to thermal sources. Due to their technical characteristics, LEDs have a great potential for use in biotechnologies for the cultivation of edible and medicinal mushrooms [38,42].

It was experimentally found that blue light is a key signaling component that regulates gene expression and globally rebuilds cellular metabolism in fungi [33,34]. In the cultivation of edible macrofungi, blue light is used to stimulate the growth of fruiting bodies. Arjona et al. [37] reported that blue light induces \textit{Pleurotus ostreatus} morphogenesis. Nakano et al. [43] demonstrated the dependence of the growth phase of \textit{P. ostreatus} mycelium using LED on light intensity. Feng et al. [44] reported that irradiation with blue light using LED increased the yield of fruiting bodies of \textit{Pholiota nameko} and \textit{Pleurotus eryngii}, Huang et al. [41] reported that when using blue LED in the cultivation of \textit{Lentinus savor-caju} not only the yield and nutritional value of fruiting bodies increased, but also the antioxidant activity of extracts of fruiting bodies increased.

Based on the results of our study, we observed an increase in the synthesis of mycelial mass and polysaccharides by the \textit{H. erinaceus} IBK 977 strain under LED irradiation in all modes used. The maximum amount of mycelial mass and polysaccharides were obtained in the range $\lambda=470$ nm, $\lambda=488$ nm, $\lambda=650$ nm. In addition, under these regimes, a decrease in the duration of the lag phase was observed. The use of green light ($\lambda=530$ nm) proved to be less effective. It has been shown that under submerged cultivation of \textit{Pleurotus}, \textit{Ganoderma}, and \textit{Lentinus} species, there is a clear correlation between the increased amount of biomass and the synthesis of both EPS and IPS [40,45]. According to the results of our research analyses, photoinduced growth stimulation in \textit{H. erinaceus} was also accompanied by the synthesis of polysaccharides, which is assimilated from other researchers’ data.

The effect of illumination with light in the blue, green, and yellow wavelength ranges during the period of vegetative growth and formation of primordia on the formation of fruiting bodies, morphology, and antioxidant activity of \textit{Pleurotus eryngii} and \textit{Hypsizygus marmoreus} was studied [46]. Illumination of vegetative mycelium with green light induced fruiting in \textit{H. marmoreus} [47], while illumination of \textit{L. edodes} in this range for 30 days after inoculation of substrate blocks was ineffective. Araújo et al. [48] investigated the effect of green light on the growth of mycelial mass and the activity of extracellular enzymes of species of the genus \textit{Pleurotus} under submerged cultivation conditions. LED served as the light source, as in our studies. During the experiment, it was found that green light reduced the growth of mycelial biomass of all species studied but increased the cellulolytic and xylanolytic activities. The cellulolytic activity of most strains increased in the presence of green light, ranging from 1.5 to 8 times depending on the mushroom species. Green light reduces laccase activity for most strains.

According to the results of our study, when using green light ($\lambda=530$ nm), an increase in the synthesis of mycelial mass and EPS by the \textit{H. erinaceus} IBK 977 was noted. The same trend was observed when analyzing the number of IPS in the mycelial mass. However, green light illumination of \textit{H. erinaceus} mycelium proved to be the least effective compared to blue and red light.

In the scientific literature available, there are no data on the effect of low-intensity light on the fatty acid profile of the mycelial mass of \textit{H. erinaceus}. Considering that light affects the metabolism of fungi, we studied the effect of irradiation with different wavelengths on the fatty acid profile of the mycelial mass of \textit{H. erinaceus} IBK 977. The presence of a significant amount of PUFA, including essential ones, in the composition of the obtained mycelial mass allows us to classify it as a useful source of lipids [49]. According to the literature, consuming unsaturated fatty acids, especially...
long-chain polyunsaturated fatty acids, is necessary to reduce blood cholesterol levels and regulate cellular physiology [50,51]. Several fatty acids, such as oleic, arachidonic, and linoleic acids, contained in the mycelial mass are essential for human metabolism [52].

The fact that heptadecenoic acid increases upon irradiation at 530 nm deserves special attention. It has important pharmacological properties and functions as an antiinflammatory and decongestant compound, and it is also active in psoriasis, allergies, and autoimmune diseases, especially in their prevention [53]. In addition, the mycelial mass of *H. erinaceus* contains IPS that have biological activity with proven beneficial properties for the human body, which can enhance the nutraceutical effect of this species.

As a result of our study, it was shown that irradiation with low-intensity quasimonochromatic light affects the fatty acid profile of the mycelial mass of *H. erinaceus* IBK 977. Changes occurred both in the quantitative and qualitative composition of the components. The predominance of PUFA over MUFA and an overall low fat content indicate that *H. erinaceus* is a desirable product in dietary regimens where controlled fat intake is required. According to Barroetaveña and Toledo [54], the predominance of PUFA over MUFA and SFA has also been reported in other basidiomycete species.

From the point of view of fungal metabolism, fatty acids are important as aromatic precursors; for example, linoleic acid is a precursor of the specific fungal alcohol 1-octen-3-ol [55]. From PUFA, according to a certain pattern, carbonyl compounds are formed, giving different aromatic compounds [56]. Depending on the carbon atom of PUFA at which the oxidative reaction occurred, first hydroperoxides and then aromatic components of various chemical properties are formed. Fatty acid derivatives as a group of volatile aromatic components have been described for a large number of plants [57]. There is an opinion that the taste of *H. erinaceus* is very similar to chicken and seafood (shrimp, crabs, lobsters). In the fruit bodies, 32 aromatic substances were found, significantly exceeding the similar properties of *Lentinus edodes*. A change in the enzymatic activity, the occurrence of oxidative processes during *H. erinaceus* light irradiation can lead to a more intense release of the aroma or its modification. For example, the fermentation of *H. erinaceus* with lactic acid bacteria has allowed the development of beverages with better nutritional and sensory qualities [58]. A new type of soy sauce was developed with the addition of *H. erinaceus* because it showed antimicrobial properties, significantly contributed to the accumulation of volatile components (esters and alcohols), and had a positive effect on the organoleptic component [59]. Therefore, further study of the correlation between changes in the fatty acid composition of *H. erinaceus* and aromatic characteristics is the goal of our further research.

Thus, the results of studies of photoreactions in *H. erinaceus* allow us to speak about the possibility of implementing effective mycobiotechnologies using LED and laser of a certain wavelength. That may have broad prospects for the targeted regulation of the biosynthetic activity of the strain-producer of the mycelial mass and biologically active substances.

**CONCLUSIONS**

The analysis of the data we have obtained allows us to confidently consider low-intensity quasimonochromatic light in the selected modes as a promising environmentally friendly stimulant for the biosynthesis of mycelial biomass, polysaccharides, and unsaturated fatty acids in *H. erinaceus* IBK 977. Furthermore, studying the photobiological reactions of fungi and accumulating experimental data on the light regulation of their biosynthetic activity can bring us closer to understanding the fundamental mechanisms of how light affects mushrooms.

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**Author contributions:** O. Mykchaylova planned the work, investigated growth and contributed to the article’s conception, made critical revisions to the manuscript, and conducted a study of the effect of low-intensity light on the dynamics of the accumulation of the mycelial mass of *H. erinaceus* IBK 977 under submerged cultivation, designed the figures and tables. M. Lomberg performed the literature review and article writing and editing, translated the text of the manuscript into English, conducted a study of the effect of low-intensity light on the dynamics of the polysaccharides’ accumulation of *H. erinaceus* IBK 977 under submerged cultivation, designed the figures and tables. G. Dubova investigated the effect...
of low-intensity light on the fatty acid profile of the mycelial mass of *H. erinaceus* IBK 977. A. Negriyko defined the light sources design and its modes of operations, calculated the doses of irradiation of the *H. erinaceus* IBK 977 inoculum, carried out its irradiation, discussed the irradiation effects, and participated in the paper preparation. N. Poyedinok planned the work, obtained and provided the new literature data for review, coordinated the work, and made critical revisions to the manuscript. All authors read and approved the final version of the manuscript.

**Conflict of interest disclosure:** The authors declare no conflict of interest.

**Data availability:** Data underlying the reported findings are deposited in the NCBI database available at: https://www.ncbi.nlm.nih.gov/nuccore/MN646239.1. Publicly available datasets were analyzed in this study. This data can be found here: https://www.gbif.org/occurrence/2580369446. Data underlying the reported findings (also) were provided as a raw dataset which is available here: https://www.serbiosoc.org.rs/NewUploads/Uploads/Mykhaylova%20et%20al_Dataset.pdf

**REFERENCES**


SUPPLEMENTARY MATERIAL

**Supplementary Table S1.** Calculation parameters of the lighting system.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wavelength</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>λ=470 nm</td>
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<tr>
<td>Energy of a photon, J·10⁻¹⁹</td>
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<tr>
<td>Value of the visibility function</td>
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<td>Number of diodes</td>
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<tr>
<td>Luminous flux from one diode, lm</td>
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<tr>
<td>Radiant power of the diode, mW</td>
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<td>Total luminous flux from all the diodes, lm</td>
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<td>Illuminance, lx (lm/m²)</td>
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<tr>
<td>Light intensity on the surface, W/m²</td>
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<tr>
<td>Photon flux density, photons/m²s.</td>
<td>7.4E+19</td>
</tr>
<tr>
<td>Photon flux density, μE/m²s.</td>
<td>122</td>
</tr>
</tbody>
</table>
Supplementary Fig. S1. Scheme of research on the detection of low-intensity quasimonochromatic light on growth and the biosynthetic activity of *Hericium erinaceus* IBK 977: I – reseeding of *H. erinaceus* IBK 977 mother culture; II – culture homogenization and inoculation of Erlenmeyer flasks; cultivation of inoculum under submerged culture conditions; III – irradiation of flasks with mycelium with quasimonochromatic light in different wavelength regimes; IV – inoculation of Erlenmeyer flasks with irradiated mycelium and growing on an orbital shaker; V – separation of the mycelial mass from the culture liquid; VI – determination of the fatty acid profile of the mycelial mass under different irradiation regimes; VII – determination of the influence of quasimonochromatic light on the dynamics of mycelial mass growth and the content of intracellular polysaccharides; VIII – determination of the amount of extracellular polysaccharides in the culture liquid.