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Effects of cultivation parameters on intracellular polysaccharide production in submerged culture of the edible medicinal mushroom *Lentinula edodes*

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The effect of different submerged culture conditions (medium composition, pH and temperature) on mycelium biomass and production of intracellular polysaccharides (IPS) by the valuable edible and medicinal mushroom *Lentinula edodes* was investigated. In total, 12 carbon and 7 nitrogen sources in the nutrient medium were tested.

Glucose and peptone were determined to be the most suitable carbon and nitrogen sources, respectively, both for IPS and biomass production. Generally, modified glucose-peptone medium, C/N ratio of 18, pH adjusted to 6.0 and cultivation temperature of 25 °C were found to enhance the yield of biomass and IPS by mycelium of *L. edodes* IBK 2541 in submerged culture. Selection of medium composition and physical cultivation parameters resulted in an IPS production of 940 mg/l on the modified glucose-peptone medium, which is three-fold higher than that from the basal medium (306 mg/l).

Key words: endopolysaccharides, biomass, shiitake, submerged fermentation, medium composition, temperature, pH.

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Práce přináší výsledky studia vlivu různých podmínek (složení média, pH a teploty) na tvorbu mycelia a produkci intracelulárních polysacharidů (IPS) při pěstování *Lentinula edodes* v submersní kultuře. Celkem bylo testováno 12 různých zdrojů uhlíku a 7 zdrojů dusíku v živném médiu.

Jako nejvhodnější zdroj uhlíku byla stanovena glukóza, jako nejvhodnější zdroj dusíku pepton, a to jak pro produkci biomasy, tak IPS. V rámci testovaných zdrojů a hodnot vychází jako nejlepší pro zvýšení tvorby biomasy a produkce IPS testovaného kmene *L. edodes* IBK 2541 modifikované glukózo-peptonové medium s poměrem C/N 18 při kultivační teplotě 25 °C a pH 6. Při tomto složení média a zvolených parametrech kultivace bylo dosaženo produkce IPS 940 mg/l, což je třikrát více než bylo zjištěno při kultivaci v základním médiu (306 mg/l).

INTRODUCTION

Mycelium and fruitbodies of many mushrooms (Basidiomycota) have been reported to possess a large number of biologically active substances, such as polysaccharides, triterpenes, phytohormones, etc. (Wasser 2002, Al-Maali et al. 2016, Vedenicheva et al. 2018). *Lentinula edodes* (Berk.) Pegler, commonly known as shiitake, is now the world's leading cultivated edible mushroom with about 22% of the world's supply (Royse et al. 2017). Moreover, numerous bioactive compounds have been isolated from fruitbodies, mycelium and liquid culture medium of *L. edodes* (Wasser 2002, Xu et al. 2014, Ruthes et al. 2016, Gaitán-Hernández et al. 2019). The biological activity of most medicinal macrofungi is largely determined by compounds of a carbohydrate nature, which comprise up to 60% of the dry biomass (Wasser 2002, Chen et al. 2012, Mizuno & Nishitani 2013). Carbohydrates are represented by free and bound sugars, mono- to polysaccharides. Polysaccharides are the best known mushroom-derived substances which perform energy-reserving, regulatory, osmoregulatory and structural functions. The intense research on bioactive carbohydrates produced by mushrooms and search for their potential sources started in the late 1960s, after a group of Japanese scientists discovered the oncostatic effect of polysaccharides isolated from the fruitbodies of *L. edodes* (Chihara et al. 1969).

The most famous and best studied intracellular polysaccharide is a linear β-(1,3)-glucan, which has the common name 'lentinan' and was isolated from *L. edodes* (Dennert & Tucker 1973). Numerous researches have reported that this compound has strong antitumour and immunomodulation properties (Chihara et al. 1987, Ina et al. 2013, Zhang et al. 2018). Liu et al. (2019) showed that this IPS promotes intestinal health, partly through altering intestinal microbiota composition and increasing the short-chain fatty acid synthesis, which subsequently leads to a reduction in inflammation. Also in this work it was demonstrated that IPS inhibited inflammatory signalling pathways (toll-like receptor 4 and nucleotide binding oligomerisation domain protein) and pro-inflammatory cytokines (tumour necrosis factor-α, interleukin-1β and interleukin-6) expression. Moreover,

the possibility of IPS to reduce the level of cytokines was noted by Zi et al. (2020). Thus it was shown in this work that IPS significantly reduced interleukin-8 and chemokine ligand-2 mRNA. In addition, these authors found that lentinan can increase the activity of superoxide dismutase and glutathione peroxidase, being approximately 18- and 2.7-fold higher, respectively, compared to the control. In general, IPS isolated from mycelium and fruitbodies of *L. edodes* have numerous properties such as antibacterial activity in vitro against gram-positive and gram-negative bacteria (Ishikawa et al. 2001, Rasmy et al. 2010), antioxidant activity (You et al. 2011, Huang et al. 2012), cardiovascular activity (Wasser & Weis 1999), liver protective activity (Akamatsu et al. 2004), anti-inflammatory activity (Lindequist et al. 2005), radioprotective activity (Pillai & Devi 2013), antidiabetic activity (Dubey et al. 2019) and others.

Subsequently, more data about antitumour activity of polysaccharides from *L. edodes* have been reported. Therefore, the research on their biological activity has been extended (Maeda & Chihara 1973, Hamuro & Chihara 1985, Giavasis 2014, Wang et al. 2017). Thanks to this, it has been demonstrated that various polysaccharides obtained from shiitake, including lentinan, exhibited an antitumour effect against colon cancer cells (Wang et al. 2017), induced mitochondrial-mediated apoptosis in human cervical carcinoma HeLa cells (Ya 2017), and enhanced the effect of oxaliplatin under hepatocellular carcinoma therapy (Zhang et al. 2016). Furthermore, shiitake extracts are reported to have immunomodulatory activity (Mizuno & Nishitani 2013). From the mycelium and liquid culture extracts of this medicinal mushroom, several pharmaceutical preparations have been developed, containing lentinan and eritadenine, which are used for the treatment of suppressed immune function, cancer, allergy, influenza, common cold, heart disease, hyperlipidemia, hypertension, hepatitis and urinary inconsistencies (Bisen et al. 2010).

Since recently, submerged cultivation has been used in the production of mycelium and bioactive compounds from mycelium biomass and liquid culture medium (Kim et al. 2002, Babitskaya et al. 2004, Elisashvili et al. 2004, Scherba & Babitskaya 2008, Krasnopolskaya et al. 2012, Elisashvili 2012). The submerged fermentation procedure is considered more advantageous for the production of polysaccharides than obtaining these products from fruitbodies (Kim et al. 2002, Bisko et al. 2018, Mykchaylova et al. 2019). This cultivation technology provides the possibility to select operating parameters for the efficient production of extracellular and intracellular polysaccharides. An analysis of literature data about submerged fungus cultivation has provided information on different aspects of exopolysaccharide synthesis (Kim et al. 2002, Babitskaya et al. 2004, Elisashvili et al. 2004, Scherba & Babitskaya 2008). Fewer works deal with intracellular polysaccharides (Elisashvili 2012). Krasnopolskaya et al. (2012) have reported on the optimisation of nutrient medium for submerged cultivation

of the selected strain of *L. edodes* with a biomass yield and endopolysaccharide content up to 21 g/l and 4.8 g/l of culture liquid, respectively. Scherba & Babitskaya (2008) have demonstrated that some strains of *L. edodes* and some other fungi grown under submerged conditions synthesise up to 8 g/l exopolysaccharides and 8–10 g per 100 g biomass of endopolysaccharides, suggesting their production potential.

The efficiency of the biotechnological process is determined mainly by the liquid nutrient medium, as well as by the nutritional substances, and availability of carbon and nitrogen sources. Furthermore, polysaccharide productivity depends on the biological characteristics of the strain and on cultivation conditions (Scherba et al. 1999, Elisashvili 2012, Krasnopolskaya et al. 2012).

The objective of our research was to evaluate the effects of the main nutrient sources (carbon and nitrogen) and their ratio, temperature and pH on the production of biomass and intracellular polysaccharide (IPS) content in the mycelium of *L. edodes* IBK 2541 during submerged cultivation.

MATERIAL AND METHODS

Strain and medium. The studied strain of *Lentinula edodes* IBK 2541 was obtained from the IBK Mushroom Culture Collection of the M.G. Kholodny Institute of Botany NASU (Bisko et al. 2016a, 2016b). This strain was selected from the IBK Collection after preliminary screening of 51 strains on the basis of maximum IPS content in submerged conditions (Mustafin et al. 2018, and unpublished data). The composition of the basal glucose-peptone nutrient medium was as follows (g/l): glucose 10.0; peptone 2.0; KH_2PO_4 1.0; K_2HPO_4 1.0; $MgSO_4 \cdot 7H_2O$ 0.25; corn steep liquor (Sigma-Aldrich) 20 ml; distilled water 1.0 l; pH 5.5 after sterilisation.

Inoculum preparation and cultivation conditions. The inoculum was initially prepared by cultivating *L. edodes* IBK 2541 mycelium for 7 days at 25 ± 0.1 °C on a basal glucose-peptone medium containing 20 g/l agar-agar. Using the obtained inoculum, the basal medium was inoculated (10% v/v) with the mycelium homogenised for 3 × 30 seconds in a laboratory blender. Cultivation was conducted on a laboratory shaker under the following conditions: temperature 25 ± 0.1 °C, agitation speed 120 rpm, volume 50 ml of basic glucose-peptone medium in 250 ml Erlenmeyer flasks for 4 days. For the fermentation stage, the inoculum obtained as described above was used for inoculation of the nutrient medium (10% v/v) in a series of experiments on the effect of various physical factors and conditions (temperature, pH, carbon and nitrogen sources, their ratio) on the biomass production and IPS yield in mycelium of *L. edodes* IBK 2541. Cultivation was carried out on a laboratory shaker for 7 days (120 rpm) in

250 ml Erlenmeyer flasks (for each trial 5 repetitions were made) containing 50 ml of nutrient medium.

Determination of biomass and IPS. After filtration of the cultural liquid, the mycelium was washed with distilled water and dried to absolutely dry biomass (a.d.b.) at 105 °C. For IPS extraction, the whole mycelium obtained from one flask was dried at 60 °C and homogenised in a laboratory blender, then supplemented with distilled water (1:10 by weight) and boiled in a water bath (100 ± 1 °C) for 18 hours. Cytoplasmic contents were removed by multiple centrifugations (3,000 g for 15 minutes) of the homogenised mycelium suspended in distilled water. The washing procedure was stopped only when optical density of the supernatant at 280 nm did not exceed 0.1 (Goncharova et al. 1996).

The obtained extracts were concentrated two- or three-fold with a rotary evaporator (60 °C), treated with 96% ethanol (volume ratio 1:1) at a temperature of 4 ± 0.1 °C and allowed to stand until complete precipitation. The precipitate (IPS fractions) was separated by centrifugation and then dialysed against distilled water for 3 days. The dialysed IPS was precipitated with ethanol (volume ratio 1:2), washed with ethanol, ether, acetone and dried at 37 °C. The IPS content was calculated in % of absolutely dry biomass.

The IPS production (mg/l) was calculated as the total amount of endopolysaccharides per litre of medium consumed for the cultivation of mycelium over the entire period of cultivation.

Effects of carbon, nitrogen sources and their ratio. The economic coefficient (EC) of using glucose as a carbon source for IPS content was calculated with the following formula:

$$EC\ (\%) = M / (C_{initial} - C_n) \times 100$$

where M is IPS mass (g/l); $C_{initial}$ and C_n are glucose concentrations (g/l) in the medium before cultivation and at time t_n after cultivation, respectively. The amount of glucose in the nutrient medium was determined by the phenol-sulphuric acid method (Grushenko et al. 1978).

The effect of different glucose concentrations (10, 20, 30, 40, 50 g/l) in the nutrient medium on biomass accumulation and IPS content in the mycelium was estimated.

Various carbon sources equivalent to 10 g/l glucose were added to the basal glucose-peptone medium. The following carbohydrates or sugar alcohols were used as carbon sources: glucose, arabinose, xylose, galactose, mannose, fructose, lactose, maltose, sucrose, mannitol, sorbitol and cellobiose.

Various nitrogen sources equivalent to the amount of nitrogen in 2 g/l peptone were added to the basal glucose-peptone medium. Inorganic (sodium nitrate,

ammonium nitrate, ammonium chloride, ammonium sulphate) and organic (asparagine, urea, peptone) compounds were used as nitrogen sources.

The effect of the carbon to nitrogen (C/N) ratio in the nutrient medium on IPS production by mycelium of the studied strain was investigated on a glucose-peptone medium containing 30 g/l glucose, with peptone additions ranging from 2 g/l to 4.7 g/l. The C/N ratio in the nutrient medium varied from 42 to 18.

Effect of medium pH. The effect of pH values on IPS production by mycelium of *L. edodes* IBK 2541 was studied on a nutrient medium with selected concentrations of carbon and nitrogen sources and C/N ratio. The medium pH was adjusted to the required level (3.0–7.5) with 10% KOH and 1M HCl. Measurements of the initial medium pH values were carried out after sterilisation in an autoclave (1 atm).

Effect of temperature. The effect of different cultivation temperatures (20, 25, 30 ± 0.1 °C) on biomass accumulation and IPS content in mycelium of *L. edodes* IBK 2541 was studied on a nutrient medium with selected concentrations of carbon and nitrogen sources, C/N ratio and pH.

Statistical analysis. All data were processed using statistical analysis methods. Values of standard deviations, coefficients of variation, and confidence intervals were calculated using standard statistical packages, Microsoft Office Excel and StatSoft Statistica 6.0. Experimental data from quintuple measurements was expressed as mean ± SD. The Student's *t*-test was applied for expressing the significance; values at $P < 0.05$ were considered significant. Results provided in tables and figures are presented as means ± standard deviation ($n = 5$).

RESULTS AND DISCUSSION

Carbon sources

Medium composition is known to affect the yield of mycelium and polysaccharides. In order to identify the best carbon sources for mycelial growth and IPS production by *Lentinula edodes* IBK 2541, twelve carbohydrates were tested. The data represented in Fig. 1 indicate that the mushroom was capable of using all the tested carbon sources. Among the sources examined, glucose (6.8 g/l) followed by cellobiose (6.5 g/l), maltose (6.4 g/l) and lactose (6.2 g/l) resulted in the best mycelial growth, whereas the biomass yield reached 5.8 and 3.2 g/l in the presence of sucrose and mannitol, respectively.

Analysing the data obtained, we noted that of all the tested compounds, IPS content was higher in the cultures cultivated in the medium with glucose (4.5% of the biomass), followed by cellobiose (3.5%) and lactose (3.2%), whereas sorbitol

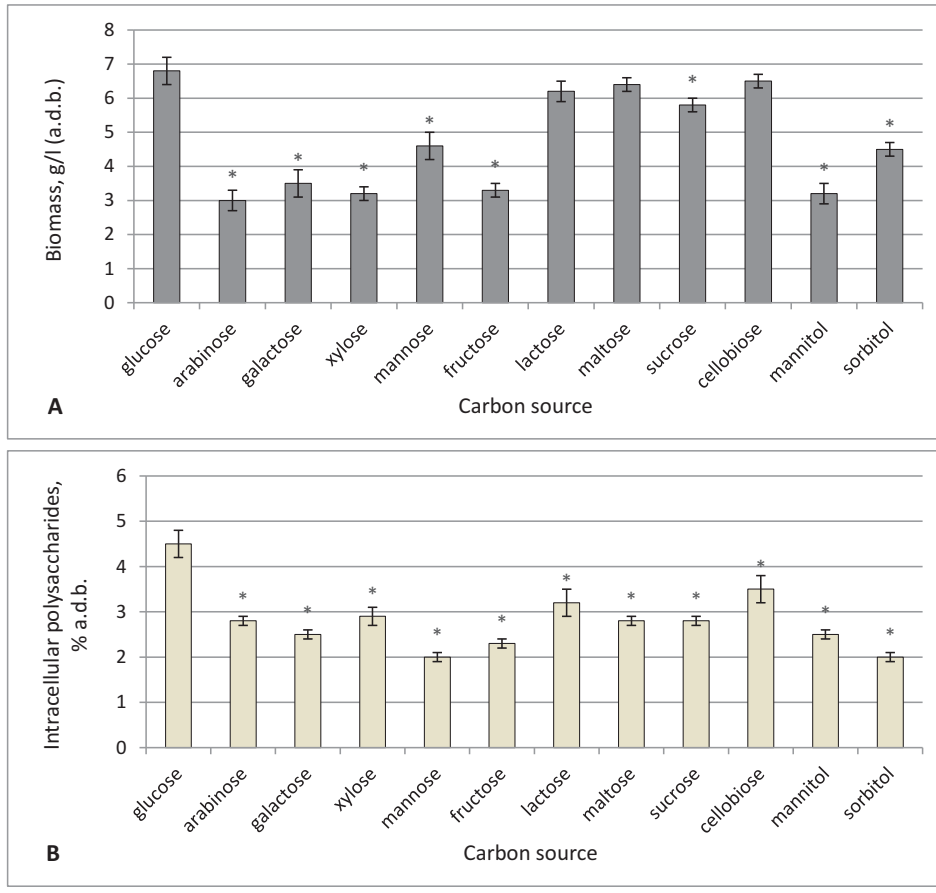

Fig. 1. Effect of various carbon sources in basal nutrient medium on biomass production (A) and intracellular polysaccharide content (B) of *Lentinula edodes* IBK 2541 under submerged cultivation. Different carbon sources were added to a basal medium equivalent to 10 g/l glucose.
* $p < 0.05$ in comparison with corresponding biomass production and IPS content in medium with glucose. Values are given as mean ± SD (n = 5).

and mannose rendered the lowest yield of IPS (2.0%). Thus, glucose was the preferred carbon source for IPS production by *L. edodes* IBK 2541. In this case, the IPS production in the medium containing glucose was 306 ± 31 mg/l (see Fig. 1: biomass 6.8 g/l contained 4.5% IPS). Krasnopolskaya et al. (2012) reported that in their study, glucose was also the best carbon source for IPS production. Therefore, glucose was used as a carbon source in our further experiments.

The effect of different glucose concentrations ranging from 10 to 50 g/l was tested. It was found that the highest IPS content occurred in the glucose-peptone

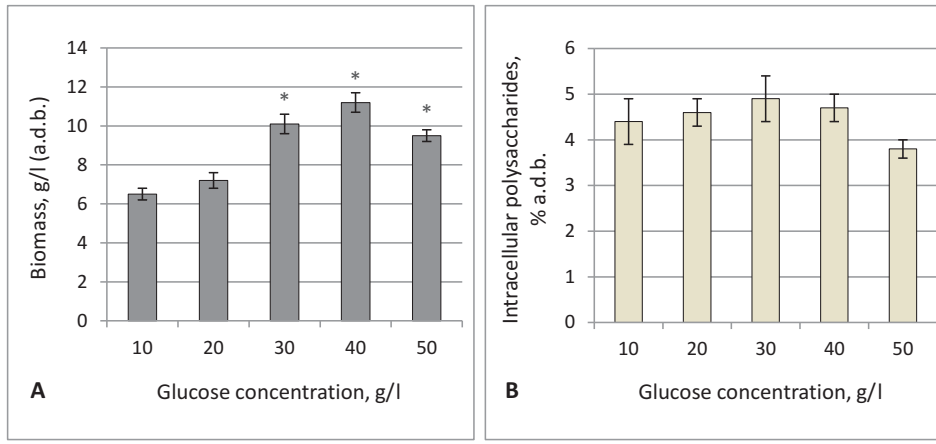

Fig. 2. Effect of glucose concentration in glucose-peptone medium on biomass production (A) and intracellular polysaccharide content (B) of *Lentinula edodes* IBK 2541 under submerged cultivation. * $p < 0.05$ in comparison with corresponding biomass production and IPS content in medium with 10 g/l glucose. Values are given as mean ± SD (n = 5).

medium with a glucose concentration of 30 g/l. It should be noted that an increase in glucose concentration from 10 g/l to 40 g/l did not significantly affect the IPS synthesis, but had an essential effect on biomass accumulation by *L. edodes* IBK 2541. In the medium containing 30 g/l of glucose, the amount of biomass was up to 55% higher as compared to the biomass obtained with the initial glucose concentration of 10 g/l. Moreover, higher biomass production under a glucose concentration of 30 g/l generally reflected growing IPS production, which increased to 494 ± 45 mg/l (see Fig. 2: biomass 10.1 g/l contained 4.9% IPS). However, at a higher glucose concentration (40 g/l), the fermentation response was not in that proportion. Although an increase in glucose concentration to 40 g/l had a positive effect on biomass yield, there was no obvious correlation between biomass and IPS content as compared to the glucose concentration of 30 g/l (Fig. 2). At the same time, this would raise the costs of the preparation of the nutrient medium.

The different authors assumed that different carbon sources might have different effects. The information reported by Elisashvili et al. (2004) on the influence of carbon and nitrogen sources in the nutrient media on the biomass of two strains of *L. edodes* is in accordance with our results. In that study, seven carbon sources were tested for their effect on mushroom growth and extracellular polysaccharide formation. However, fungal growth and polysaccharide production greatly depended on the compound used in the nutrition medium. All the fungi showed their highest mycelia dry weights when cultivated in a medium supplemented with cellobiose, mannitol or glucose. Thus, the biomass of *L. edodes* strains accounted

for 5.0–5.8 g/l. A much lower final biomass was found after fungi cultivation in the presence of xylose or sucrose (Elisashvili et al. 2004). Krasnopolskaya et al. (2012) reported that on the basic medium containing glucose (20 g/l) and soy flour, the highest biomass yield for the selected strain of *L. edodes* was 8–12 g/l. Krupodorova et al. (2019) indicated that among the tested carbon sources, the highest mycelium yield of *L. edodes* was observed for glucose, followed by cellulose. Glucose was also reported as a best carbon source in an *L. edodes* cultivation with a biomass accumulation of 2.75 g/l to 6.88 g/l (Feng et al. 2010).

Nitrogen sources

Another factor essential for efficient mushroom growth and polysaccharide production is the nitrogen source used for fungi cultivation. The effect of different investigated organic and inorganic nitrogen sources on biomass production and IPS content by *L. edodes* IBK 2541 when grown on 30 g/l glucose was assessed. The results of our study on seven nitrogen sources indicated that peptone, asparagine and ammonium sulphate are the most favourable for maximum biomass production by *L. edodes* IBK 2541, yielding 6.5, 6.2 and 6.0 g/l, respectively (Fig. 3).

In the study by Elisashvili et al. (2004) six nitrogen sources were tested. Of these, corn steep liquor yielded the highest mycelia growth with 8.4 g/l, as well as peptone with 7.7 g/l. Krupodorova et al. (2019) studied another strain of *L. edodes*, IBK 502, showing that asparagine was the best source of nitrogen for biomass production but ammonium sulphate and peptone were found to be the worst by them.

In the present study, the IPS production by various nitrogen sources ranged from 2.4 to 4.5 g/l. Analysis of IPS contents in biomass demonstrated that peptone was the best nitrogen source for IPS production by *L. edodes* IBK 2541 (Fig. 3). Adding asparagine as nitrogen source to a medium significantly decreased the IPS content in the biomass as compared to medium containing peptone. The least IPS was recorded in urea-containing medium. Our data demonstrated that the biomass growth and IPS content were not much higher on media with other nitrogen sources in the basal nutrient medium (sodium nitrate, ammonium nitrate, ammonium chloride and urea), as shown in Fig. 3.

Since C/N ratio is crucial for mushroom growth in submerged cultivation (Elisashvili 2012), we set different C/N ratios by changing only nitrogen (peptone) concentrations, with carbon concentrations (glucose) fixed at 30 g/l in the media.

In order to increase biomass production and IPS content, the C/N ratio was manipulated from 18 to 42. The results of biomass production, IPS content, residual content of glucose and economic coefficient (EC) at different C/N ratios are provided in Tab. 1.

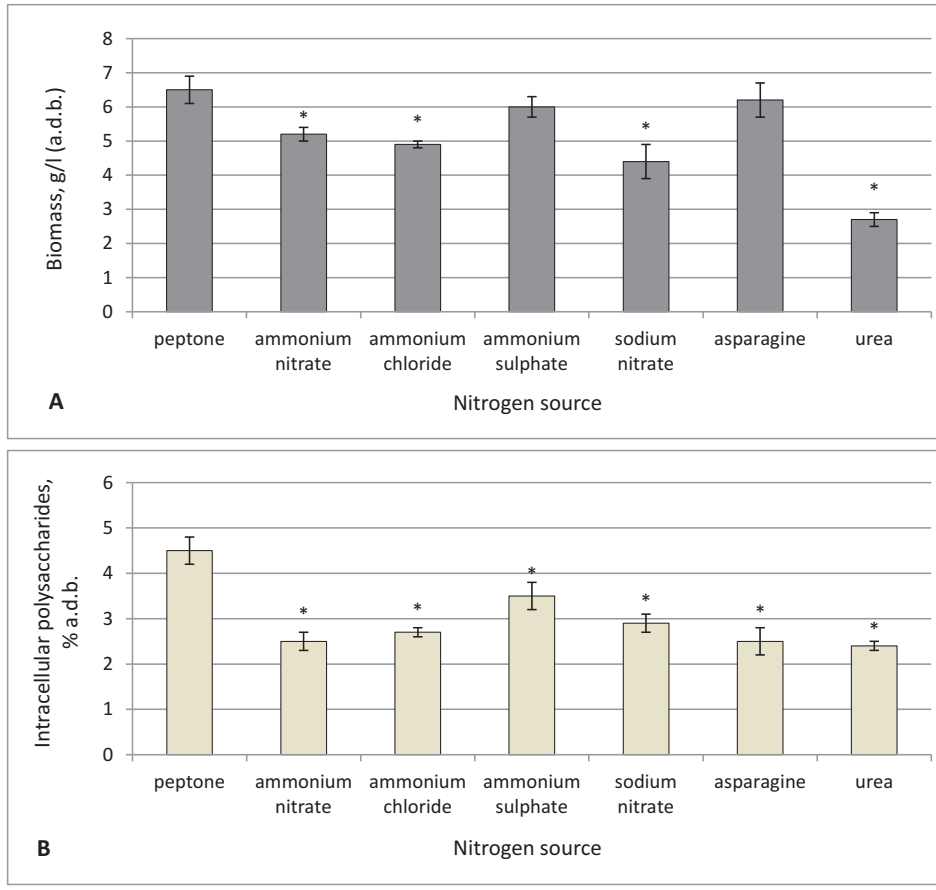

Fig. 3. Effect of various nitrogen sources in basal nutrient medium on biomass production and intracellular polysaccharide content of *Lentinula edodes* IBK 2541 under submerged cultivation. Various nitrogen sources were added to a basal medium equivalent to 2 g/l peptone.
* $p < 0.05$ in comparison with corresponding biomass production and IPS content in medium with peptone. Values are given as mean ± SD (n = 5).

As seen from Tab. 1, with a gradual increase in peptone content in the medium from 2 g/l (C/N = 42) to 4.7 g/l (C/N = 18), an increase in biomass and IPS content was observed. Among the C/N ratios tested, the C/N ratio of 18:1 yielded the highest IPS production, up to 724 ± 37 mg/l. As for carbon consumption by mycelium *L. edodes* IBK 2541, at C/N ratio of 18:1 it was the most complete.

Tab. 1. Effect of C/N ratio in glucose-peptone medium on biomass production and IPS content of *Lentinula edodes* IBK 2541 under submerged cultivation.

C/N	Biomass (g/l)	Residual content of glucose in the medium (% initial content)	IPS (% absolutely dry biomass)	Production of IPS (mg/l)	Economic coefficient of IPS production (% used glucose)
18	10.5 ± 0.4	7	6.9 ± 0.4	724 ± 37	2.7
25	9.8 ± 0.4	26*	6.2 ± 0.3	607 ± 25*	2.5
29	8.3 ± 0.3*	48*	5.0 ± 0.4*	415 ± 26*	2.6
42	6.5 ± 0.4*	52*	4.5 ± 0.3*	293 ± 17*	2.2

* $p < 0.05$ in comparison with corresponding parameters at C/N ratio of 18. Values are given as mean ± SD (n = 5).

Effect of pH

The pH of fermentation media can greatly affect membrane function and cell growth, which may subsequently influence physiological activity and metabolite production. The results of our experiments demonstrate that *L. edodes* IBK 2541 in submerged culture is able to grow in a wide range of initial pH values of the nutrient medium (Tab. 2). In the medium with selected carbon (glucose) and nitrogen (peptone) sources and C/N ratio, a high pH was beneficial for biomass production and IPS content. As shown in Tab. 2, the optimum pH value for biomass yield was found to be in the range of 5.0–7.0. The maximum IPS production (829 ± 55 mg/l) was achieved at pH 6.0.

The differences between the strains were expressed in their relation to the pH of the nutrient medium. For strain *L. edodes* IBK 502, the maximum biomass was accumulated at the initial pH of the nutrient medium, 3.5 (Krupodorova et al. 2019). Among *L. edodes* strains IBK 57, 704, 711, 712, 717, the optimum pH for growth appeared to be between 4.5 and 5.2. The yield of dry biomass was up to 6.1 g/l after 7–10 days of submerged cultivation (Lomberh et al. 2002). During mycelial growth, pH dropped to 3.5, which limited further growth. Solomko & Mitropolskaja (1994) studied the conditions of submerged cultivation of other strains of *L. edodes*, IBK 55, 57 and 65, and proposed to use liquid nutritional media with an optimum pH of 4.5–5.0. Also, these authors showed that IBK 57 accumulated a maximum of 4.5 g of biomass per litre on the 14th day of cultivation on a medium with glucose and asparagine. After replacing the nitrogen source (ammonium sulphate instead of asparagine), all the studied strains grew better. On the 9th day of cultivation, the biomass concentration was 8–9 g/l. They also reported that the factor limiting the rate of biomass production is the pH of the medium, which in the process of cultivation decreased to 3.0–3.05 and inhibited further *L. edodes* growth.

In experiments by other authors, the optimum pH of liquid medium for maximum biomass production of *L. edodes* under submerged cultivation was indicated to be 5.5–6.0 (Lomberg & Solomko 2012), 6.0 (Elisashvili et al. 2004) or 6.2 (Krasnopolskaya et al. 2012).

Tab. 2. Effect of medium pH on biomass and IPS production of *Lentinula edodes* IBK 2541 under submerged cultivation.

Initial pH	Final pH	Biomass (g/l)	Production of IPS (mg/l)	IPS (% absolutely dry biomass)
3	3.2	4.5 ± 0.2*	140 ± 7*	3.1 ± 0.2*
4	3.3	7.1 ± 0.3*	461 ± 16*	6.5 ± 0.2
5	3.4	10.0 ± 0.5	690 ± 34	6.9 ± 0.4
6	3.5	10.5 ± 0.4	829 ± 55*	7.9 ± 0.2*
6.5	3.6	9.9 ± 0.5	643 ± 28	6.5 ± 0.3
7	3.7	9.2 ± 0.4	488 ± 18*	5.3 ± 0.2*
7.5	4.0	7.8 ± 0.4*	374 ± 16*	4.8 ± 0.2*

* $p < 0.05$ in comparison with corresponding parameters at medium pH 5. Values are given as mean ± SD ($n = 5$).

Effect of temperature

Another important factor for efficient mushroom growth and polysaccharide production is the incubation temperature. It is reported that the differences between the *L. edodes* strains are expressed in their relation to the temperature of mycelium cultivation (Lomberh et al. 2002, Lomberg & Solomko 2012, Krupodorova et al. 2019).

The influence of temperature on the growth of *L. edodes* 2541 in the nutrient medium with selected composition (see above) and pH 6.0 is shown in Fig. 4. The obtained results indicate that the highest mycelial growth and IPS yield by *L. edodes* IBK 2541 was recorded at a temperature of 25 ± 0.1 °C. The IPS production at this temperature was 940 ± 0.61 mg/l (see Fig. 4: biomass 11.6 g/l containing 8.1% IPS). Analysing the data obtained, we recorded that temperatures of 20 °C and 30 °C were less effective for its biomass and IPS production. But the scale with 5-degree intervals, which used in our experiment, is probably too wide, as Krupodorova et al. (2019) reported that in their research indicated 26–28 °C was found to be the optimal temperature for other strains of *L. edodes*.

Thus, as an output of our research, nutrient medium composition and conditions for submerged cultivation to enhance biomass production and IPS content by mycelium of *L. edodes* IBK 2541 were selected. The composition of the modified glucose-peptone medium contains the following components (g/l): glucose

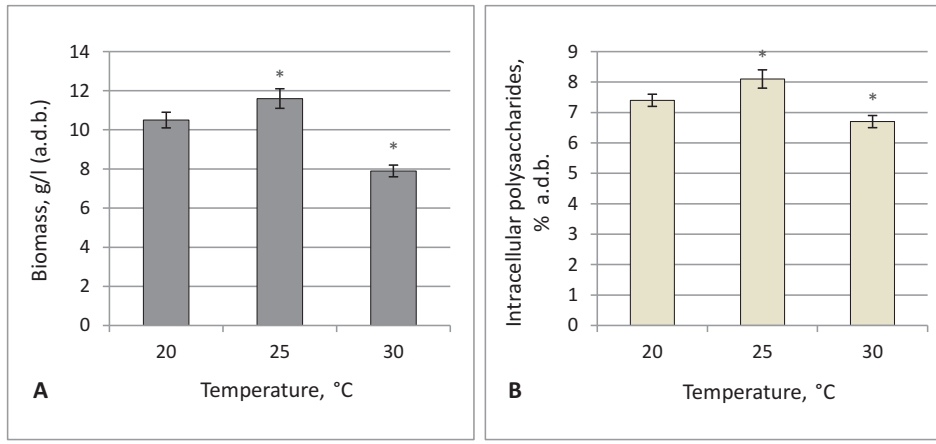

Fig. 4. Effect of temperature on biomass production (A) and intracellular polysaccharide content (B) of *Lentinula edodes* IBK 2541 under submerged cultivation.
* $p < 0.05$ in comparison with corresponding biomass production and IPS content at 20 °C. Values are given as mean ± SD (n = 5).

30.0; KH_2PO_4 1.0; K_2HPO_4 1.0; $MgSO_4$ 0.25; peptone 4.7; corn steep liquor 20 ml. The pH value of the nutrient medium is 6.0, cultivation temperature is 25 ± 0.1 °C.

CONCLUSION

An analysis of the literature and our data presented here indicates a significant effect of the biological properties of the valuable edible medicinal mushroom *Lentinula edodes* strains and conditions (medium composition, pH, temperature) on the processes of growth and IPS yield in biomass accumulation. Based on the above, to obtain the desired product, IPS, it is advisable to choose the basic parameters of the cultivation of a certain strain of *L. edodes*. Our results indicate that the proposed consistent selection of carbon sources, nitrogen, and their ratio in the nutrient medium, the pH of the nutrient medium and the temperature of cultivation result in a three-fold increase in IPS production by the mycelium of *L. edodes* IBK 2541, specifically from 306 mg/l to 940 mg/l.

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REFERENCES

AKAMATSU S., WATANABE A., TAMESADA M., NAKAMURA R., HAYASHI S., KODAMA D., KAWASE M., YAGI K. (2004): Hepatoprotective effect of extracts from *Lentinus edodes* mycelia on dimethylnitrosamine-induced liver injury. – Bio Pharm. Bull. 27(12): 1957–1960. DOI: https://doi.org/10.1248/bpb.27.1957

AL-MAALI G.A., BISKO N.A., OSTAPCHUK A.N. (2016): The effect of zinc citrate and zinc sulfate on the growth and biomass composition of medicinal mushroom *Ganoderma lucidum*. – Mikol. Fitopatol. 50(5): 313–317.

BABITSKAYA V.G., BISKO N.A., SCHERBA V.V., MITROPOLSKAYA N.YU., PUCHKOVA T.A. (2004): Some physiological aspects of the submerged cultivation of culinary-medicinal shiitake mushroom *Lentinus edodes* (Berk.) Singer (Agaricomycetideae). – Int. J. Med. Mushrooms 6(4): 369–374. DOI: https://doi.org/10.1615/IntJMedMushr.v6.i4.70

BISEN P.S., BAGHEL R.K., SANODIYA B.S., THAKUR G.S., PRASAD G.B.K.S. (2010): *Lentinus edodes*: a macrofungus with pharmacological activities. – Curr. Med. Chem. 17(22): 2419–2430. DOI: https://doi.org/10.2174/092986710791698495

BISKO N.A., LOMBERG M.L., MYTROPOLSKA N.YU., MYKCHAYLOVA O.B. (2016a): IBK Mushroom Culture Collection. – 120 p., M.G. Kholodny Institute of Botany, Alterpres, Kyiv.

BISKO N.A., MYTROPOLSKA N.Y., MYKCHAYLOVA O.B., LOMBERG M.L., AL-MAALI G.A. (2018): The rare and biotechnologically important mushroom species in the IBK collection. – In: Kłaczewski W., Stefanek H., Gorzel M., eds., Development of natural sciences in countries of the European Union taking into account the challenges of XXI century, pp. 21–37. Vincent Pol University, Lublin & Baltija Publishing, Riga.

BISKO N.A., SUKHOMLYN M.M., MYKCHAYLOVA O.B., LOMBERG M.L., TSVYD N.V., PETRICHUK YU.V., AL-MAALI G.A., MYTROPOLSKA N.YU. (2016b): Ex situ conservation of rare and endangered species in mushroom culture collections of Ukraine. – Ukr. Bot. J. 75(4): 338–347. DOI: https://doi.org/10.15407/ukrbotj75.04.338

CHEN H., JU Y., LI J., YU M. (2012): Antioxidant activities of polysaccharides from *Lentinus edodes* and their significance for disease prevention. – Int. J. Bio. Macromol. 50(1): 214–218. DOI: https://doi.org/10.1016/j.ijbiomac.2011.10.027

CHIHARA G., HAMURO J., MAEDA Y.Y., SHIIO T., SUGA T., TAKASUKA N., SASAKI T. (1987): Antitumor and metastasis-inhibitory activities of lentinan as an immunomodulator: an overview. – Cancer Detect. Prev. Suppl. 1: 423–443.

CHIHARA G., MAEDA Y., HAMURO J., SASAKI T., FUKUOKA F. (1969): Inhibition of mouse sarcoma 180 by polysaccharides from *Lentinus edodes* (Berk.) Sing. – Nature 222(5194): 687–688. DOI: https://doi.org/10.1038/222687a0

DENNERT G., TUCKER D. (1973): Antitumor polysaccharide Lentinan – a T cell adjuvant. – Journal of the National Cancer Institute 51(5): 1727–1729. DOI: https://doi.org/10.1093/jnci/51.5.1727

DUBEY S.K., CHATURVEDI V.K., MISHRA D., BAJPEYEE A., TIWARI A., SINGH M.P. (2019): Role of edible mushroom as a potent therapeutics for the diabetes and obesity. – 3 Biotech 9(12), 450. DOI: https://doi.org/10.1007/s13205-019-1982-3

ELISASHVILI V. (2012): Submerged cultivation of medicinal mushrooms: bioprocesses and products (review). – Int. J. Med. Mushrooms 14(3): 211–239. DOI: https://doi.org/10.1615/intjmedmushr.v14.i3.10

Elisashvili V., Wasser S., Tan K.K., Chichua D., Kachlishvili E. (2004): Extracellular polysaccharide production by culinary-medicinal shiitake mushroom *Lentinus edodes* (Berk.) Singer and *Pleurotus* (Fr.) P. Karst. species depending on carbon and nitrogen source. – Int. J. Med. Mushrooms 6(2): 165–172. DOI: https://doi.org/10.1615/IntJMedMushr.v6.i2.70

Feng Y.L., Li W.Q., Wu X.Q., Cheng J.W., Ma S.Y. (2010): Statistical optimization of media for mycelial growth and exo-polysaccharide production by *Lentinus edodes* and a kinetic model study of two growth morphologies. – Biochem. Eng. J. 49: 104–112. DOI: https://doi.org/10.1016/j.bej.2009.12.002

Gaitán-Hernández R., López-Peña D., Esqueda M., Gutiérrez A. (2019): Review of bioactive molecules production, biomass, and basidiomata of shiitake culinary-medicinal mushrooms, *Lentinus edodes* (Agaricomycetes). - Int. J. Med. Mushrooms 21(9): 841–850. DOI: https://doi.org/10.1615/IntJMedMushrooms.2019031849

Giavasis I. (2014): Bioactive fungal polysaccharides as potential functional ingredients in food and nutraceuticals. – Curr. Opin. Biotechnol. 26: 162–173. DOI: https://doi.org/10.1016/j.copbio.2014.01.010

Goncharova I.A., Scherba V.V., Babitskaya V.G. (1996): Polisacharidy kletochnoj stenki bazidiomiceta *Coriolus hirsutus* [Polysaccharides of the cell wall of basidiomycete *Coriolus hirsutus*]. – Prikl. Biokhim. Mikrobiol. 32(4): 434–437. [in Russian]

Grushenko M.M., Anikeenko T.S., Reznikov V.M. (1978): Sovmestnoe ispol'zovanie fenol-sernokislogo i toluidinovogo sposobov opredeleniya sakharov kak metod izucheniya uglevodnogo sostava lignouglevodnogo kompleksa [The joint use of phenol-sulphuric acid and toluidine methods for the determination of sugars as a method for studying the carbohydrate composition of the ligno-carbohydrate complex]. – In: Lignouglevodnye kompleksy drevesiny [Lignocarbohydrate complexes of wood], pp. 32–35. Zinatne, Riga. [in Russian]

Hamuro J., Chihara G. (1985): Lentinan, a T-cell oriented immunopotentiator: its experimental and clinical applications and possible mechanism of immune modulation. – In: Fehiche R.L., Chirigos M.A., eds., Immune modulation agents and their mechanisms, pp. 409–436. Marcel Dekker, New York.

Huang X., Tu Z., Jiang Y., Xiao H., Zhang Q., Wang H. (2012): Dynamic high pressure microfluidization-assisted extraction and antioxidant activities of lentinan. – Int. J. Biol. Macromol. 51(5): 926–932. DOI: https://doi.org/10.1016/j.ijbiomac.2012.07.018

Ina K., Kataoka T., Ando T. (2013): The use of lentinan for treating gastric cancer. – Anticancer Agents Med. Chem. 13(5): 681–688. DOI: https://doi.org/10.2174/1871520611313050002

Ishikawa N.K., Kasuya M.C.M., Vanetti M.C.D. (2001): Antibacterial activity of *Lentinula edodes* grown in liquid medium. – Braz. J. Microbiol. 32(3): 206–210. DOI: https://doi.org/10.1590/S1517-83822001000300008

Kim S.W., Hwang H.J., Park J.P., Cho Y.J., Song C.H., Yun J.W. (2002): Mycelial growth and exo-biopolymer production by submerged culture of various edible mushrooms under different media. – Lett. Appl. Microbiol. 34(1): 56–61. DOI: https://doi.org/10.1046/j.1472-765x.2002.01041.x

Krasnopolskaya L.M., Kats N.Yu., Usov A.I., Barkov A.V., Vinokurov V.A. (2012): Pogruzhennoe kultivirovanie shtamma bazidiomiceta *Lentinus edodes* s shirokim spektrom biologicheskoj aktivnosti [Submerged cultivation of *Lentinus edodes* strain with broad spectrum of biological activity]. – Antibiotics and Chemotherapy 57: 3–7. [in Russian]

Krupodorova T.A., Barshteyn V.Yu., Kizitska T.O., Pokas E.V. (2019): Effect of cultivation conditions on mycelial growth and antibacterial activity of *Lentinula edodes* and *Fomitopsis betulina*. – Czech Mycol. 71(2): 167–186. DOI: https://doi.org/10.33585/cmy.71204

Lindequist U., Niedermeyer T.H.J., Jülich W.D. (2005): The pharmacological potential of mushrooms. – Evid. Based Complement. Alternat. Med. 2(3): 285–299. DOI: https://doi.org/10.1093/ecam/neh107

Liu Y., Zhao J., Zhao Y., Zong S., Tian Y., Chen S., Li M., Liu H., Zhang Q., Jing X., Sun B., Wang H., Sun T., Yang C. (2019). Therapeutic effects of lentinan on inflammatory bowel disease and colitis-associated cancer. – J. Cell. Mol. Med. 23(2), 750–760. DOI: https://doi.org/10.1111/jcmm.13897

LOMBERG M.L., SOLOMKO E.F. (2012): Rost kul'tur makromicetov na agarizovannyh pitatel'nyh sredah i plotnyh substratah [Growth of the macromycetes cultures on agar nutrient media and solid substrates]. – In: Wasser S.P., ed., Biological features of medicinal macromycetes in culture, Vol. 2, pp. 345–371. Alterpres, Kyiv. [in Russian]

LOMBERH M.L., SOLOMKO E.F., BUCHALO A.S., KIRCHHOFF B. (2002): Studies of medicinal mushrooms in submerged cultures. – In: Montiel E., Huerta G., Sanchez H.E., eds., Mushroom biology and mushroom products. Proceedings of the 4th International Conference on Mushroom Biology and Mushroom Products, pp. 367–378, Mexico.

MAEDA Y.Y., CHIHARA G. (1973): The effects of neonatal thymectomy on the antitumour activity of lentinan, carboxymethylpachymaran and zymosan, and their effects on various immune responses. – Int. J. Cancer 11(1): 153–161. DOI: https://doi.org/10.1002/ijc.2910110118

MIZUNO M., NISHITANI Y. (2013): Immunomodulating compounds in Basidiomycetes. – J. Clin. Biochem. Nutr. 52(3): 202–207. DOI: https://doi.org/10.3164/jcbn.13-3

MUSTAFIN K., BISKO N.A., NARMURATOVA ZH.B., ZHAKIPBEKOVA A.S. (2018): Screening of *L. edodes* strains producing biomass with content of polysaccharides. – Reports of the National Academy of Sciences of the Republic of Kazakhstan 4(320): 22–26.

MYKCHAYLOVA O.B., LOMBERG M.L., BISKO N.A. (2019): Verification and screening of biotechnologically valuable macromycetes species *in vitro*. – In: Jankovska A., ed., Development of modern science: the experience of European countries and prospects for Ukraine, pp. 354–375. Baltija Publishing, Riga. DOI: https://doi.org/10.30525/978-9934-571-78-7_51

PILLAI T.G., DEVI U.P. (2013): Mushroom beta glucan: potential candidate for post irradiation protection. – Mutat. Res. 751(2): 109–115. DOI: https://doi.org/10.1016/j.mrgentox.2012.12.005

RASMY G.E., BOTROS W.A., KABEIL S., DABA A.S. (2010): Preparation of glucan from *Lentinula edodes* edible mushroom and elucidation of its medicinal value. – Australian Journal of Basic and Applied Sciences 4(11): 5717–5726.

ROYSE D.J., BAARS J., TAN Q. (2017): Current overview of mushroom production in the world. – In: Zied D.C., Pardo-Giménez A., eds., Edible and medicinal mushrooms: Technology and applications, 1st ed., pp. 5–13. Wiley, Chichester. DOI: https://doi.org/10.1002/9781119149446.ch2

RUTHES A.C., SMIDERLE F.R., IACOMINI M. (2016): Mushroom heteropolysaccharides: A review on their sources, structure and biological effects. – Carbohydr. Polym. 136: 358–375. DOI: https://doi.org/10.1016/j.carbpol.2015.08.061

SCHERBA V.V., BABITSKAYA V.G., TRUCHONOVEC V.V., FOMINA V.I., BISKO N.A., MITROPOLSKAYA N.YU. (1999): The influence of the cultivation conditions on the chemical composition of medicinal mushrooms *Pleurotus ostreatus* (Jacq.: Fr.) Kumm. and *Lentinus edodes* (Berk.) Sing. – Int. J. Med. Mushrooms 1(2): 181–185. DOI: https://doi.org/10.1615/IntJMedMushrooms.v1.i2.80

SCHERBA V.V., BABITSKAYA V.G. (2008): Polysaccharides of xylotrophic basidiomycetes. – Appl. Biochem. Microbiol. 44(1): 78–83. DOI: https://doi.org/10.1134/S0003683808010134

SOLOMKO E.F., MITROPOLSKAJA N.YU. (1994): Poluchenie posevnogo materiala *Lentinus edodes* (Berk.) Sing. glubinnym metodom [Obtainance of seed material of *Lentinus edodes* (Berk.) Sing. by the deep cultivation method]. – Mikol. Fitopatol. 28(3): 34–39. [in Russian]

VEDENICHEVA N.P., AL-MAALI G.A., BISKO N.A., SHCHERBATIUK M.M., LOMBERG M.L., MYTROPOLSKA N.Y., MYKCHAYLOVA O.B., KOSAKIVSKA I.V. (2018): Comparative analysis of cytokinins in mycelial biomass of medicinal mushrooms. – Int. J. Med. Mushrooms 20(9): 837–847. DOI: https://doi.org/10.1615/IntJMedMushrooms.2018027797

WANG J., LI W., HUANG X., LIU Y., LI Q., ZHENG Z., WANG K. (2017): A polysaccharide from *Lentinus edodes* inhibits human colon cancer cell proliferation and suppresses tumor growth in athymic nude mice. – Oncotarget 8(1): 610–623. DOI: https://doi.org/10.18632/oncotarget.13481

WASSER S.P. (2002): Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. – Appl. Microbiol. Biotechnol. 60: 258–274. DOI: https://doi.org/10.1007/s00253-002-1076-7

Wasser S.P., Weis A.L. (1999): Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: current perspectives (review). – Int. J. Med. Mushrooms 1(1): 31–62. DOI: https://doi.org/10.1615/IntJMedMushrooms.v1.i1.30

Xu X.F., Yan H.D., Tang J., Chen J., Zhang X.W. (2014): Polysaccharides in *Lentinus edodes*: Isolation, structure, immunomodulating activity and future prospective. – Crit. Rev. Food Sci. Nutr. 54: 474–487. DOI: https://doi.org/10.1080/10408398.2011.587616

Ya G. (2017): A *Lentinus edodes* polysaccharide induces mitochondrial-mediated apoptosis in human cervical carcinoma HeLa cells. – Int. J. Biol. Macromol. 103: 676–682. DOI: https://doi.org/10.1016/j.ijbiomac.2017.05.085

You R., Wang K., Liu J., Liu M., Luo L., Zhang Y. (2011): A comparison study between different molecular weight polysaccharides derived from *Lentinus edodes* and their antioxidant activities *in vivo*. – Pharm. Biol. 49(12): 1298–1305. DOI: https://doi.org/10.3109/13880209.2011.621960

Zhang Y., Li Q., Wang J., Cheng F., Huang X., Cheng Y., Wang K. (2016): Polysaccharide from *Lentinus edodes* combined with oxaliplatin possesses the synergy and attenuation effect in hepatocellular carcinoma. – Cancer Letters 377(2): 117–125. DOI: https://doi.org/10.1016/j.canlet.2016.04.037

Zhang Y., Zhang M., Jiang Y., Li X., He Y., Zeng P., Guo Z., Chang Y., Luo H., Liu Y., Hao C., Wang H., Zhang G., Zhang L. (2018): Lentinan as an immunotherapeutic for treating lung cancer: a review of 12 years clinical studies in China. – J. Cancer Res. Clin. Oncol. 144(11): 2177–2186. DOI: https://doi.org/10.1007/s00432-018-2718-1

Zi Y., Jiang B., He C., Liu L. (2020): Lentinan inhibits oxidative stress and inflammatory cytokine production induced by benzo(a)pyrene in human keratinocytes. – J. Cosmet. Dermatol. 19(2): 502–507. DOI: https://doi.org/10.1111/jocd.13005