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Effect of cultivation conditions on mycelial growth and antibacterial activity of *Lentinula edodes* and *Fomitopsis betulina*

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This research dealt with the effect of cultivation conditions on mycelial growth and antibacterial activity of *Lentinula edodes* and *Fomitopsis betulina* in liquid static culture. The antibacterial activity of mycelium and culture liquid were assayed against the bacteria *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* using the disk diffusion method.

The optimum incubation period of mycelial growth for both species and their antibacterial activity was 14 days. The biomass of the studied species was the largest at temperatures of 26–28 °C. The highest mycelium production was recorded at pH 3.5–4.0, whereas the strongest antibacterial activity was observed at pH 5.5.

Of the carbon sources tested, the highest mycelium productivity of *L. edodes* was recorded for glucose, followed by cellulose. Cellulose was found to be the optimal carbon source for the highest antibacterial activity of *L. edodes*. Cellulose was also the optimal carbon source for mycelial growth in *F. betulina*, but the highest antibacterial activity was achieved with added galactose. For both fungal species, asparagine was found to be the nitrogen source producing a maximum biomass, whereas the highest antibacterial activity of *L. edodes* and *F. betulina* was noticed after adding ammonium nitrate and peptone, respectively.

The antibacterial potential of culture liquids appeared to be higher in the studied species when compared to their mycelium. Establishing optimum cultivation conditions will allow for obtaining maximum productivity of mycelium and culture liquids with the highest antibacterial activity.

Key words: xylotrophic basidiomycetes, shiitake, *Piptoporus*, fungal growth conditions, fungal antibiotic activity.

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Krupodorova T.A., Barshteyn V.Yu., Kizitska T.O., Pokas E.V. (2019): Vliv kultivačních podmínek na růst mycelia a antibakteriální aktivitu *Lentinula edodes* a *Fomitopsis betulina*. – Czech Mycol. 71(2): 167–186.

Studie sleduje vliv kultivačních podmínek na růst mycelia a antibakteriální aktivitu *Lentinula edodes* a *Fomitopsis betulina* ve staticky kultivované tekuté kultuře. Antibakteriální aktivita mycelia a tekutiny z kultury byla testována proti *Bacillus subtilis*, *Staphylococcus aureus* a *Escherichia coli* s využitím diskové difuzní metody.

Optimální inkubační doba pro růst mycelia obou druhů a jejich antibakteriální aktivitu je 14 dní, Největší nárůst biomasy sledovaných druhů byl zjištěn shodně při teplotách 26–28 °C. Nejvyšší produkce mycelia byla zaznamenána při pH 3,5–4,0, zatímco vrcholná antibakteriální aktivita byla pozorována při pH 5,5.

Nejlepším zdrojem uhlíku pro produkci mycelia *L. edodes* se jeví glukóza, jako druhý nejlepší zdroj pak celulóza. Ta je oproti tomu optimálním zdrojem uhlíku pro antibakteriální aktivitu *L. edodes* a taktéž pro růst mycelia *F. betulina*, zatímco nejvyšší antibakteriální aktivita březovníku byla zjištěna při přidání galaktózy. Jako nejlepší zdroj dusíku pro produkci biomasy obou druhů byl zjištěn asparagin, zatímco nejvyšší antibakteriální aktivita byla u *L. edodes* zaznamenána při přidání dusičnanu amonného a u *F. betulina* při přidání peptonu do kultivačního média.

Ve srovnání s mycelium vykazuje vyšší antibakteriální potenciál tekutina z kultury sledovaných druhů. Nastavení optimálních podmínek kultivace umožní získat maximální produkci mycelia a tekutiny z kultur s nejvyšší antibakteriální aktivitou.

INTRODUCTION

The discovery of new natural sources of biologically active compounds is particularly relevant due to the emergence of multi-resistant bacterial strains, which pose a serious threat to global health. Especially basidiomycetes which are able to synthesise antibacterial metabolites are significant (Alves et al. 2012).

The antibacterial potential of the well-known edible and medicinal mushroom, *Lentinula edodes* (formerly *Lentinus edodes*), or shiitake, has been demonstrated in numerous studies. The researchers observed different degrees of antibacterial activity, including a high therapeutic effect. *Lentinula edodes* extracts showed antibacterial activity against different gram-positive and gram-negative bacteria, specifically *Bacillus subtilis* (Hassegawa et al. 2005, Soboleva et al. 2006, Casaril et al. 2011, Pauliuc & Botău 2013, Osman et al. 2014, Ponugupati 2015), *Staphylococcus aureus* (Hatvani 2001, Soboleva et al. 2006, Avci et al. 2014, Islam et al. 2015), *Escherichia coli* (Song et al. 1987, Casaril et al. 2011, Avci et al. 2014, Islam et al. 2015), or all these bacteria together (Hearst et al. 2009, Bobritskaya et al. 2013, Chowdhury et al. 2015, Han et al. 2015). However, the number of studies into antibacterial activity of *L. edodes* mycelium (Song et al. 1987, Soboleva et al. 2006, Osman et al. 2014) is incomparable to the number of studies into the antibacterial activity of fruitbodies.

Some publications have demonstrated antibacterial activity of the medicinal non-edible mushroom *Fomitopsis betulina* (formerly *Piptoporus betulinus*) (Pleszczyńska et al. 2017). Fruitbody extracts and isolated compounds were found to be active against *S. aureus*, *B. subtilis* (Dresch et al. 2015, Alresly et al. 2016) and *E. coli* (Alresly et al. 2016). Antibiotic piptamine obtained from culture

liquid of *P. betulinus* Lu 9-1 inhibited growth of *B. subtilis*, *E. coli* (Schleger et al. 2000, Keller et al. 2002) and *S. aureus* (Schleger et al. 2000).

Oxalic acid from culture filtrate of *L. edodes* showed antimicrobial activity against phytopathogenic bacteria (Kwak et al. 2016). Triterpene acids (Kamo et al. 2003) and polyporenic acids (Pleszczyńska et al. 2017) isolated from *F. betulina* showed pharmacological activity, such as anti-inflammatory and anticancer activity, respectively.

Experiments studying the effect of controlled cultivation conditions on medicinal edible and/or non-edible mushroom growth, synthesis of metabolites and therapeutic properties are especially important. Biosynthetic processes necessary for mushroom cell activity are quite controllable. This allows for the regulation of growth, and of cellular and extracellular metabolite production by changing the physical and chemical parameters of cultivation (Gbolagade et al. 2006, Vieira et al. 2008, Wong et al. 2009, Manjunathan & Kaviyarasan 2010, Nwokoye et al. 2010, Kibar & Peksen 2011, Johnsy & Kaviyarasan 2013). But as far as we know, only the study by Hassegawa et al. (2005) focused on the effect of cultivation factors on antibacterial activity of *L. edodes*.

The aim of this study is thus to search for optimal cultivation conditions of the above-mentioned medicinal fungi, and to assay their antibacterial activity under different conditions.

MATERIAL AND METHODS

Source of fungi. *Lentinula edodes* (Berk.) Sing. IBK 502 and *Fomitopsis betulina* (Bull.) B.K. Cui, M.L. Han & Y.C. Dai IBK 327 were kindly supplied by the Culture Collection of Mushrooms (IBK) of the M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine.

Inoculum preparation. Mycelial cultures were grown in Petri dishes (90 mm diameter) on glucose-peptone-yeast agar culture medium (GPY) at pH 6.0, composed of (g/l): 25.0 glucose, 3.0 yeast extract, 2.0 peptone, 1.0 K_2HPO_4, 1.0 KH_2PO_4, 0.25 $MgSO_4 \cdot 7H_2O$, and 20.0 agar. Seed cultures were grown in 250 ml Erlenmeyer flasks containing 50 ml of liquid GPY medium each, sterilised by autoclaving for 20 min at 121 °C. Each flask of medium was inoculated with three mycelial plugs of 8 mm diameter cut from the Petri dishes using a sterile borer in the mycelial active growth stage.

Biomass dry weight. Mycelium was separated from the medium by filtration through Whatman's filter paper No. 4, washed with distilled water, dried to constant weight at 105 °C. Biomass of the mushrooms was determined as absolutely dry weight (a.d.w.).

Antibacterial activity. Bacterial cultures of *Bacillus subtilis* UCM B-901 (ATCC 6633), *Escherichia coli* UCM B-906 (ATCC 25922) and *Staphylococcus aureus* UCM B-4001 (ATCC 65388) were kindly supplied by the Ukrainian Collection of Microorganisms (UCM, Institute of Microbiology and Virology of the NAS of Ukraine). Tested microorganisms were cultured on Mueller Hinton agar (MHA) (37 °C, 24 h). Each microorganism was suspended in sterile saline solution and diluted to 10^6 colony forming units (CFU) per ml.

Antibacterial activity was determined using the agar disc diffusion method (Bilai 1982). Sterile paper discs (8 mm) with the tested mushroom species (mycelium homogenised by using a mortar and pestle or evaporated five times using a sand bath cultural liquid) were placed into Petri dishes with MHA previously inoculated with bacterial suspensions. The inoculated Petri dishes were incubated overnight at 37 °C. Antibacterial activity was evaluated by measuring the zone of inhibition against the tested organisms (in mm, using a ruler graduated to 0.5 mm). Antibacterial activity was considered to be present where the zone of inhibition was larger than 8 mm (exceeding diameter of the inserted paper disc). Distilled water was used as a negative control.

The effect of incubation conditions on biomass growth and antibacterial activity. In order to determine the effect of incubation time, the flasks were inoculated with seed cultures and incubated at 25 °C for different durations (7, 14, 21, 28 and 35 days). After the incubation period, the biomass was measured as dry weight, antibacterial activity was recorded using the disc-diffusion method as mentioned above.

To assess the effect of temperature, the flasks with seed cultures were incubated at different temperatures (24, 26, 28 and 30 °C) in various incubators for 14 days (chosen as optimal incubation period for maximum mycelial growth, see Results). After the incubation period, the optimal temperature was recorded by establishing the biomass dry weight as mentioned above.

To study the effect of pH level, the flasks with seed cultures were incubated for 14 days at 26 ± 1 °C at different pH levels (4.0, 4.5, 5.0, 5.5, 6.0, 6.5). The medium was adjusted to the desired pH by adding 1M HCl and 1M NaOH using a digital pH-meter before autoclaving. After the incubation period, growth as well as antibacterial activity were determined as mentioned above.

The basic medium (50 ml in 250 ml Erlenmeyer flasks), selected for the study of carbon and nitrogen nutrition, consisted of (g/l): 10.0 glucose, 0.4 asparagine, 1.0 KH_2PO_4, 0.5 $MgSO_4 \cdot 7H_2O$ (Bisko & Kosman 1988). Monosaccharides [D(–)-arabinose, D(–)-fructose, D(+)-galactose and D(+)-glucose], disaccharides (lactose, maltose and sucrose), and polysaccharides (cellulose and soluble starch) were used as carbon sources. For each of these, 4 g/l of C (pure carbon per litre calculated as the percentage of the carbon element in a molecule) were added in-

dividually to the basic medium to replace glucose. The control medium was constituted without any carbon compounds. Subsequently different amounts of galactose or cellulose, selected as the most suitable for antibacterial activity of the species in question (10, 15, 25 g), were dissolved in 1000 ml of the basic medium at pH 5.5.

Different nitrogen sources (sodium nitrate, ammonium nitrate, ammonium sulphate, urea, L-asparagine and peptone) were used for the study. For each of these, 0.21 g/l of N (pure nitrogen per litre calculated as the percentage of nitrogen element in a molecule) was added individually to the basic medium to replace asparagine. The control medium was constituted without any nitrogen compounds. As the most suitable for antibacterial activity in subsequent tests, different amounts of peptone (0.5, 1.5, 3.0, 4.5, 6.0 g) and ammonium nitrate (0.0625, 0.125, 0.25, 0.5, 0.75 g) were dissolved in 1000 ml of the basic medium.

After the incubation period (14 days at 26 ± 1 °C), growth as well as antibacterial activity were determined as mentioned above.

Statistical analysis. The experimental results were expressed as means ± SEM (standard error of the mean) of triplicates. Statistical analysis was performed using Fisher's F-test. The data was analysed with Excel statistical functions using the Microsoft Office XP software, Statistical Package for Social Sciences, version 11.5 (SPSS Inc., Chicago, 2002). Differences at $P \leq 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Optimal incubation period for mycelial growth and antibacterial activity

The incubation period of 14 days was optimal, since we did not observe a statistically significant difference in mycelial growth of both fungi after 21, 28 or 35 days (Fig. 1).

This result is quite close to the optimum incubation period for the growth of two *Lentinula edodes* strains, 15 days and 13 days of incubation for *L. edodes* LC2141 and *L. edodes* LC202, respectively (Osman et al. 2009). Many researchers have, however, reported different incubation periods for optimum *L. edodes* biomass production. Moreover, the resultant biomass yield was higher than those observed by other researches in a submerged culture of *L. edodes* strains at incubation periods of 5 to 27 days – from 1 to 5.24 g/l (Song et al. 1987, Tan & Moore 1992, Kim et al. 2002, Hassegawa et al. 2005, Aminuddin et al. 2013), and in the case of incubation without agitation (stationary culture) 4.3 g/l after 24 days (Hassegawa et al. 2005) and 3.26 g/l after 28 days (Tan & Moore 1992). The quantity of

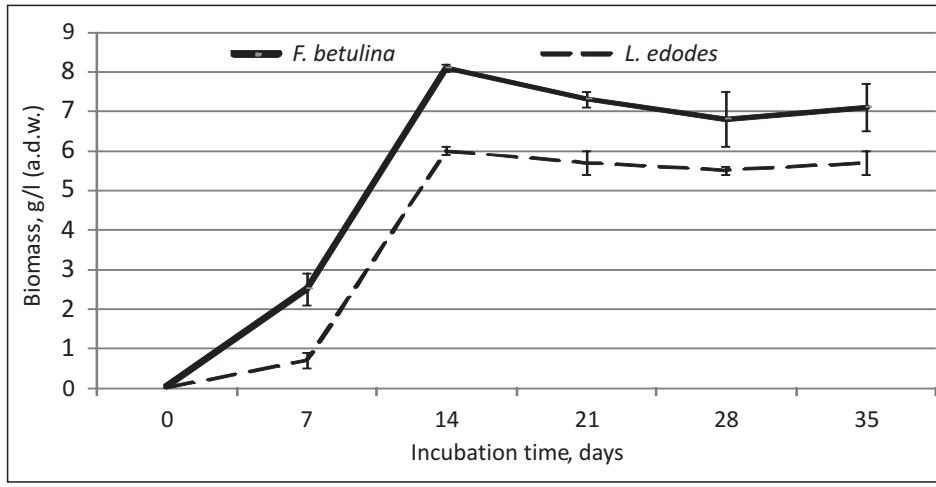

Fig. 1. Effect of incubation period on *Fomitopsis betulina* and *Lentinula edodes* biomass growth. Values are presented as means ± standard error of the mean (SEM).

L. edodes biomass was higher on mediums with sawdust after 7 days and after 14 days of cultivation in a circular shaker (Rudic & Dvornina 2001).

Antibacterial activity of *L. edodes* culture liquid remained the same (full inhibition of bacterial growth) 14 to 35 days after the start of cultivation. In *F. betulina*, the highest antibacterial metabolite production was obtained after 14 days of cultivation (Tab. 1). The antibacterial activity of the mycelium appeared only after a long period of cultivation: 35 days for *L. edodes* and 28 days for *F. betulina* (maximum activity). Although it may seem inconsistent that the highest antibacterial activity of culture liquid and mycelium was manifested at various stages of mycelium development, this may be due to the different nature of substances produced by fungi in these stages (Sazonova et al. 2013).

For the following experiments, a cultivation duration of 14 days was chosen, given the optimal incubation period for mycelial growth of both fungi species and the exhibition of antibacterial activity of culture liquid.

Optimal cultivation conditions: temperature and pH

It is well known that mycelium of mushrooms grows in both natural and artificial conditions only in a certain temperature range due to its effect on metabolic reactions. It has been reported that many species of basidiomycetes grow in a broad range of temperatures, the optimum temperature found to be 20–30 °C when tested with 5 °C intervals (Bae et al. 2000, Vahidi et al. 2004, Hassegawa et al. 2005, Gbolagade et al. 2006, Nwokoye et al. 2010, Lai et al. 2014, Dresch et al.

Tab. 1. Antibacterial activity of *Lentinula edodes* and *Fomitopsis betulina* for different incubation periods. The values represent inhibition zone diameters (mm) and are presented as means ± standard error of the mean (SEM).

Incubation period, days	*Escherichia coli*		*Bacillus subtilis*		*Staphylococcus aureus*	
	Culture liquid	Mycelium	Culture liquid	Mycelium	Culture liquid	Mycelium
Lentinula edodes						
7	12.0 ± 0.0	–	–	–	11.8 ± 0.3	–
14	**FI**	–	**FI**	–	**FI**	–
21	**FI**	–	**FI**	–	**FI**	–
28	**FI**	–	**FI**	20.0 ± 0.9	**FI**	–
35	**FI**	**FI**	**FI**	**FI**	**FI**	**FI**
Fomitopsis betulina						
7	14.8 ± 0.5	–	16.3 ± 0.5	–	19.0 ± 2.0	–
14	**FI**	–	**FI**	–	**FI**	–
21	14.0 ± 0.9	–	20.0 ± 0.6	–	**FI**	11.5 ± 0.5
28	16.0 ± 0.6	13.3 ± 1.2	15.3 ± 0.3	12.5 ± 0.9	20.0 ± 0.0	15.2 ± 0.9
35	17.7 ± 1.4	–	16.0 ± 0.7	–	19.0 ± 0.6	–

FI = full inhibition of bacterial growth (≥ 25 mm); – = lack of antibacterial activity

2015). To determine the optimal temperature for the mycelium growth of *L. edodes* and *F. betulina* they were cultivated in a narrow temperature interval. The biomass of both studied fungal species was the largest at 26–28 °C (Fig. 2). A temperature of 27 ± 1 °C was used for the next studies.

A similar result (biomass production at the same level) was obtained for *F. betulina* at 15, 20 and 25 °C (Henningsson 1965). Other authors have reported an optimal temperature of 25 °C for the growth of *L. edodes* strains (Khan et al. 1991, Kim et al. 2002, Osman et al. 2009) and isolates of *F. betulina* (Dresch et al. 2015). Hassegawa et al. (2005) reported an optimum growth temperature of 20 °C for *L. edodes*. On the other hand, in another study, the vegetative growth of *L. edodes* strains was accomplished at a temperature of 28 °C on both solid and liquid medium in static cultivation (Deepa Rani & Das 2015).

The statistical significant effect of pH of the initial medium on mycelium structure and function (in particular on cell morphology, cell membrane function, solubility of salts, and uptake of necessary nutrients) as well as on metabolite synthesis has been demonstrated for different fungal species: *Paecilomyces japonica* (Bae et al. 2000), *Ganoderma lucidum* (Fang & Zhong 2002), *Mycena leptocephala* (Vahidi et al. 2004) and *Lignosus rhinocerus* (Lai et al. 2014). The obtained results of the pH effect are consistent with studies where the mycelium of various fungal species could grow in a wide range of pH levels.

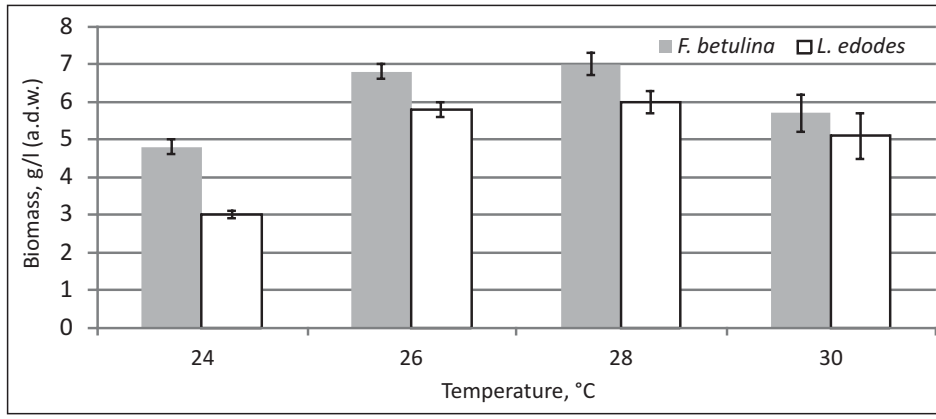

Fig. 2. Effect of various temperatures on *Fomitopsis betulina* and *Lentinula edodes* biomass growth. Values are presented as means ± standard error of the mean (SEM).

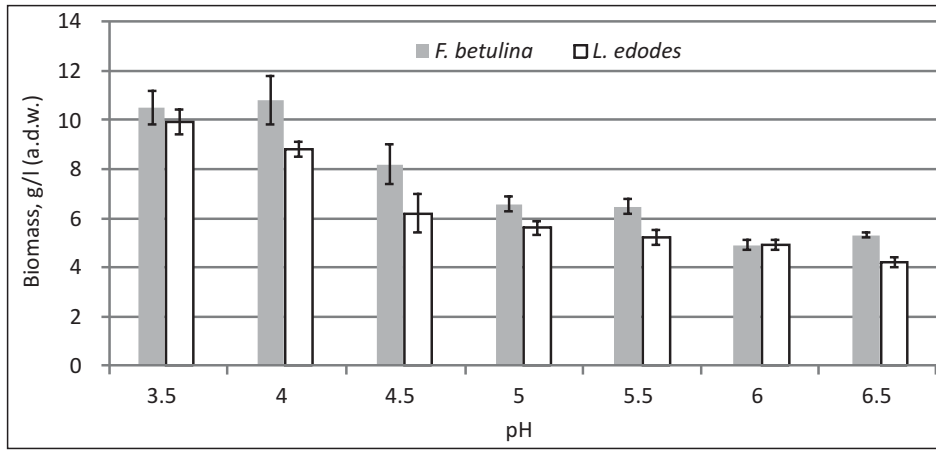

Fig. 3. Effect of pH values on *Fomitopsis betulina* and *Lentinula edodes* biomass growth. Values are presented as means ± standard error of the mean (SEM).

In our study, the influence of the studied pH levels on biomass accumulation (Fig. 3) and antibacterial metabolite production was quite similar for both fungal species (Tab. 2). Although optimal mycelium production of the studied species was observed at pH 3.5–4.0 and was statistically significantly different from biomass values at other pH levels (Fig. 3), the best antibacterial activity was established at pH 5.5. As shown in Tab. 2, the antibacterial activity of *F. betulina* culture liquid was affected more by pH value as compared to *L. edodes* culture liquid. However, although the highest values were detected at pH 5.5, no significant change in antibacterial activity was observed in the mycelium of both fungi species at different pH levels in these experiments.

Tab. 2. Antibacterial activity of *Lentinula edodes* and *Fomitopsis betulina* for different pH values. The values represent inhibition zone diameters (mm) and are presented as means ± standard error of the mean (SEM).

pH	*Escherichia coli*		*Bacillus subtilis*		*Staphylococcus aureus*	
	Culture liquid	Mycelium	Culture liquid	Mycelium	Culture liquid	Mycelium
Lentinula edodes						
3.5	10.8 ± 0.3	–	12.0 ± 0.7	–	11.2 ± 0.5	–
4.0	10.7 ± 0.3	–	10.8 ± 0.3	–	13.8 ± 0.8	–
4.5	10.0 ± 0.8	–	10.3 ± 0.3	–	11.6 ± 0.5	–
5.0	10.5 ± 0.5	–	10.5 ± 0.5	–	12.8 ± 0.6	–
5.5	13.0 ± 1.0	–	15.2 ± 0.8	–	18.5 ± 0.7	–
6.0	13.5 ± 1.1	–	14.2 ± 0.6	–	12.7 ± 0.3	–
6.5	13.5 ± 0.5	–	14.0 ± 1.0	–	10.5 ± 0.5	–
Fomitopsis betulina						
3.5	11.0 ± 0.4	–	12.5 ± 0.9	–	11.5 ± 0.5	–
4.0	10.3 ± 0.3	–	10.8 ± 0.3	–	18.3 ± 0.8	–
4.5	10.7 ± 0.5	–	10.3 ± 0.3	–	12.5 ± 1.0	–
5.0	11.5 ± 0.9	–	10.8 ± 0.5	–	12.8 ± 0.6	–
5.5	**FI**	–	18.3 ± 0.6	–	**FI**	–
6.0	**FI**	–	14.2 ± 0.6	–	20.7 ± 0.7	–
6.5	**FI**	–	–	–	**FI**	–

FI = full inhibition of bacterial growth (≥ 25 mm); – = lack of antibacterial activity

The established optimum pH was close to the one mentioned in some other studies. Komemushi et al. (1995) found that a pH of 4.0 was the optimum for *L. edodes* growth. Hassegawa et al. (2005) reported that pH 3.0–3.5 was the most favourable for the biomass production of *L. edodes*, but its best antibacterial activity was observed at pH 4.5. The best growth of *L. edodes* occurred at pH 4.3–4.8 (Kim et al. 2002) and pH 4.61 (Feng et al. 2010). In contrast, maximum mycelium growth of *L. edodes* strains was achieved at pH 6.0 (Aminuddin et al. 2013) and pH 7.0 (Osman et al. 2009). The optimal pH level for the growth of *F. betulina* in stationary liquid cultures was 3.2 (Lomberh et al. 2002). After two weeks, the growth of *F. betulina* was optimal on the medium in static cultivation with a starting pH of 5.5 (Henningsson 1965).

At the end of the incubation period, in our study, the pH level of the culture medium of both fungal species had decreased from 6.0 (in the beginning of cultivation) to 3.0–3.5. The low final pH level is similar to the results (between 3.4 and 3.8) of earlier studies into the growth of *L. edodes* (Okeke et al. 1994, Rudic & Dvornina 2001, Kim et al. 2002, Hassegawa et al. 2005) and *F. betulina* (Henningsson 1965) on different liquid mediums. The acidic pH level is generally caused by acid-producing abilities of basidiomycetes (Takao 1965).

Influence of carbon sources

Carbohydrates are one of the essential factors in mushrooms cultivation. They are structural and storage compounds in cells, and are a source of energy. The ability of mushrooms to produce various enzymes allows them to use these in a wide range of carbon sources, such as monosaccharides, disaccharides and polysaccharides. Among the tested carbon sources, the highest mycelium yield of *L. edodes* was observed for glucose, followed by cellulose. Cellulose, sucrose and glucose were favourable for the growth of *F. betulina* mycelium (Fig. 4).

One of the key issues regarding the ecology of wood-decay (xylotrophic) fungi is the understanding whether they have the ability to use carbon sources. A wood cell consists mainly of cellulose, hemicellulose and lignin. On the other hand, sucrose is a widespread reserve substance of plants formed during photosynthesis. Therefore, the good growth of the studied fungi species on cellulose, glucose and sucrose corresponds with physiological features (ability to utilise the components of the substrate) of these xylotrophs in nature. However, the ability of fungi to produce different enzymes allows them also to utilise other carbon sources. Maximum mycelium growth of *L. edodes* strains has been obtained using various carbon sources: starch (Khan et al. 1991), molasses (Tan & Moore 1992, Hassegawa et al. 2005), glucose (Kim et al. 2002, Feng et al. 2010), rice bran (Hassegawa et al. 2005), fructose (Osman et al. 2009), maltose (Petre et al. 2012), dextrose and mannitol (Deepa Rani & Das 2015). The good growth of *F. betulina* on glucose and poor utilisation of arabinose is an outcome also presented by Henningsson (1965).

In general, the antibacterial activity of *F. betulina* was more dependent on carbon sources than that of *L. edodes*. The mycelium of the studied fungus species showed a variation in diameter of the inhibition zone of the growth of the tested bacteria from 9.5 to 19.0 mm as an impact of different carbohydrates (Tab. 3). Mycelium of *L. edodes* showed antibacterial activity against *Escherichia coli* and *Bacillus subtilis* only when cellulose was used as a source of carbon. It is interesting to note that arabinose did not positively influence the growth of *F. betulina*, but supported antibacterial activity in the mycelium against all tested bacteria (Fig. 4, Tab. 3). Supplementation of maltose or starch also had an impact on the antibacterial activity of *F. betulina* mycelium against *E. coli*. Addition of galactose, maltose or sucrose resulted in antibacterial action of *F. betulina* mycelium against *Staphylococcus aureus*.

Among the tested carbon sources, cellulose and galactose were the most suitable for producing antibacterial substances by *L. edodes* and *F. betulina*, respectively (Tab. 3). Based on the results mentioned above, these carbohydrates were selected as suitable carbon sources for the following experiments.

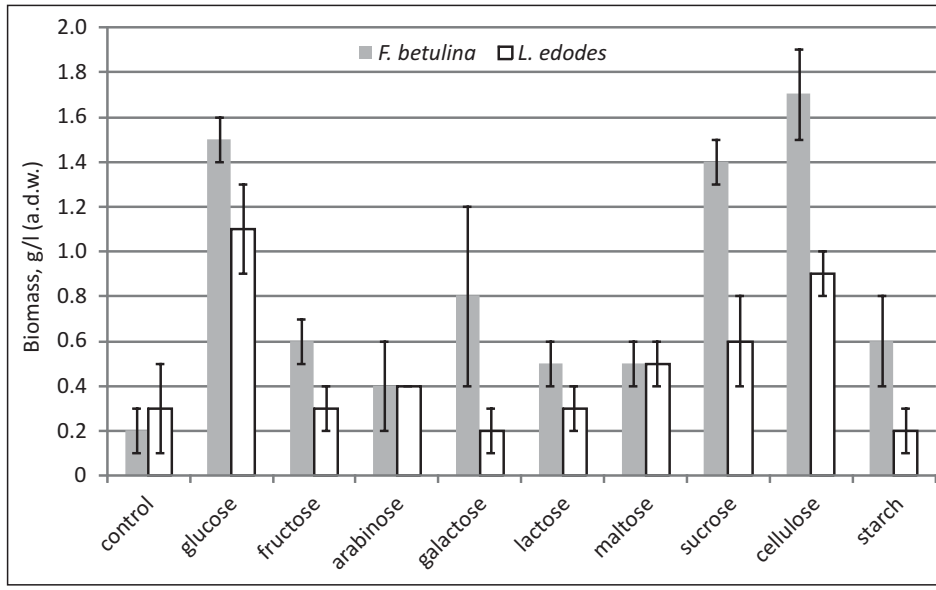

Fig. 4. Effect of carbon source on *Fomitopsis betulina* and *Lentinula edodes* biomass growth. Values are presented as means ± standard error of the mean (SEM).

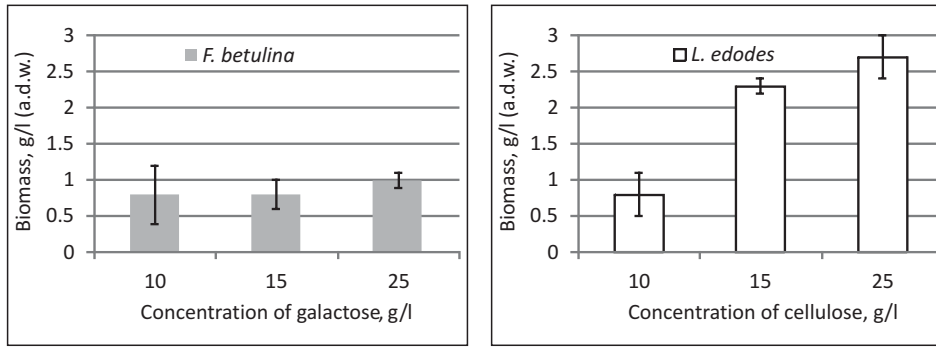

Fig. 5. Effect of carbohydrate concentration on *Fomitopsis betulina* (galactose, left) and *Lentinula edodes* (cellulose, right) biomass growth. Values are presented as means ± standard error of the mean (SEM).

It was observed that galactose concentration did not influence *F. betulina* growth in contrast to cellulose concentration in *L. edodes* cultivation (Fig. 5). However, there was no significant effect of galactose concentration on the mycelium growth of *F. betulina* (Fig. 5), in contrast to the galactose concentration effect on antibacterial activity (Tab. 5). The strongest mycelium growth of *L. edodes* was obtained when 25 g/l cellulose was used. A positive impact of carbohydrate concentration on *L. edodes* growth was also established earlier. For comparison,

Tab. 3. Antibacterial activity of *Lentinula edodes* and *Fomitopsis betulina* for different carbon sources. The values represent inhibition zone diameters (mm) and are presented as means ± standard error of the mean (SEM).

Carbon source	*Escherichia coli*		*Bacillus subtilis*		*Staphylococcus aureus*	
	Culture liquid	Mycelium	Culture liquid	Mycelium	Culture liquid	Mycelium
Lentinula edodes						
Control*	–	–	–	–	–	–
Glucose	–	–	–	–	–	–
Fructose	–	–	18.0 ± 0.0	–	13.0 ± 0.7	–
Arabinose	11.5 ± 0.5	–	–	–	13.3 ± 0.9	–
Galactose	–	–	–	–	–	–
Lactose	–	–	17.3 ± 1.4	–	–	–
Maltose	–	–	**FI**	–	–	–
Sucrose	–	–	–	–	–	–
Cellulose	**FI**	15.5 ± 0.5	**FI**	19.0 ± 0.6	**FI**	–
Starch	–	–	–	–	–	–
Fomitopsis betulina						
Control*	–	–	–	–	–	–
Glucose	–	–	11.7 ± 0.3	–	–	–
Fructose	–	–	12.0 ± 0.2	–	–	–
Arabinose	–	10.5 ± 0.3	–	11.0 ± 0.0	–	11.8 ± 0.5
Galactose	15.0 ± 0.4	–	**FI**	–	11.8 ± 0.6	14.4 ± 0.4
Lactose	12.3 ± 0.3	–	10.8 ± 0.5	–	–	–
Maltose	13.0 ± 1.0	13.0 ± 0.9	–	–	–	13.0 ± 0.9
Sucrose	13.7 ± 1.8	–	–	–	11.0 ± 0.7	12.0 ± 0.7
Cellulose	–	–	–	–	–	–
Starch	–	11.0 ± 0.4	10.5 ± 0.5	–	13.0 ± 0.9	–

FI = full inhibition of bacterial growth (≥ 25 mm); – = lack of antibacterial activity
* = without carbon source

Tab. 4. Effect of cellulose concentration on antibacterial activity of *Lentinula edodes*. The values represent inhibition zone diameters (mm) and are presented as means ± standard error of the mean (SEM).

Concentration (g/l)	*Escherichia coli*		*Bacillus subtilis*		*Staphylococcus aureus*	
	Culture liquid	Mycelium	Culture liquid	Mycelium	Culture liquid	Mycelium
10	**FI**	14.0 ± 0.3	**FI**	18.5 ± 0.5	**FI**	–
15	–	–	19.2 ± 0.7	–	–	–
25	–	–	13.0 ± 0.6	–	–	–

FI = full inhibition of bacterial growth (≥ 25 mm); – = lack of antibacterial activity

Tab. 5. Effect of galactose concentration on antibacterial activity of *Fomitopsis betulina*. The values represent inhibition zone diameters (mm) and are presented as means ± standard error of the mean (SEM).

Concentration (g/l)	*Escherichia coli*		*Bacillus subtilis*		*Staphylococcus aureus*	
	Culture liquid	Mycelium	Culture liquid	Mycelium	Culture liquid	Mycelium
10	15.1 ± 0.7	–	**FI**	–	12.4 ± 0.3	13.8 ± 0.6
15	13.3 ± 0.9	–	–	–	11.0 ± 0.0	–
25	14.5 ± 0.7	–	–	–	–	–

FI = full inhibition of bacterial growth (≥ 25 mm); – = lack of antibacterial activity

Royse et al. (1990) showed that addition of sucrose at 0.6 up to 1.2%, fructose at 1.2% and glucose at 0.6% (dry weight) to the substrate increased the yield of *L. edodes* by 11 to 20%.

The production of antibacterial metabolites of both fungi was increased when a carbon source was added to the medium in a concentration of 10 g/l (Tabs. 4, 5).

Influence of nitrogen sources

Nitrogen is another essential element, playing an important role in physiological control, regulation of metabolisms and synthesis of nitrogen-containing compounds. Among the nitrogen sources used in this study, asparagine was found to be the best nitrogen source for maximum biomass production in both fungal species (Fig. 6). Ammonium sulphate and peptone also had a positive effect on the *F. betulina* biomass yield. Compared with the *F. betulina*, mycelium growth of *L. edodes* was significantly higher with ammonium nitrate than with ammonium sulphate. The best growth for both fungi was obtained on organic nitrogen source asparagine, since the two xylotrophic species grow naturally on substrates which contain nitrogen in the form of organic compounds, preferably proteins and peptides.

However, as presented in other studies, different *L. edodes* strains needed various sources of nitrogen for their growth: urea (Khan et al. 1991), ammonium chloride (Kim et al. 2002), yeast extract and sodium nitrate (Osman et al. 2009), peptone and sodium nitrate (Deepa Rani & Das 2015). Casein hydrolysate was the most suitable for *F. betulina* growth in floating cultures, followed by ammonium salts (Henningsson 1965).

In general, our results show that the antibacterial activity of *F. betulina* appears to be more dependent on sources of nitrogen as compared to *L. edodes*. Moreover, nitrogen sources did not influence the antibacterial activity of *L. edodes* mycelium in contrast to *F. betulina* mycelium (Tab. 6). Addition of urea supported antibacterial activity of *F. betulina* mycelium against *E. coli* and *S. aureus*.

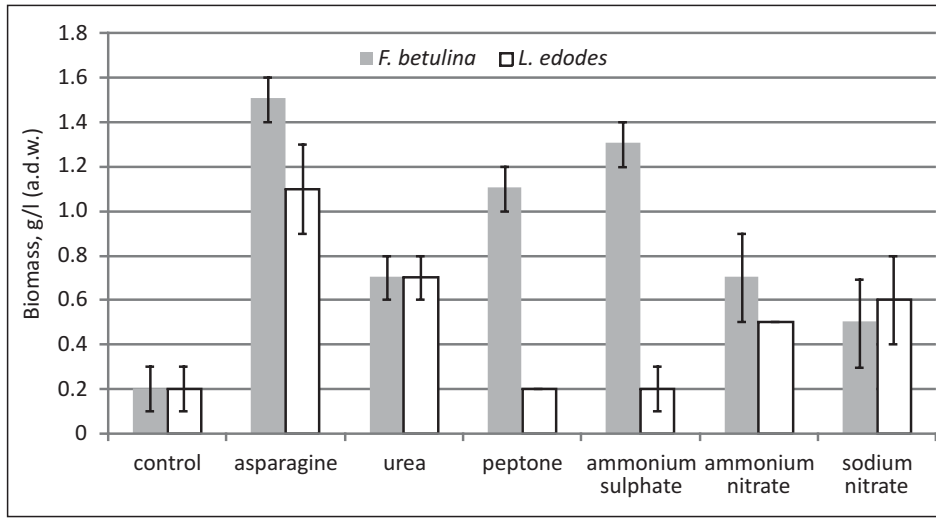

Fig. 6. Effect of nitrogen source on *Fomitopsis betulina* and *Lentinula edodes* biomass growth. Values are presented as means ± standard error of the mean (SEM).

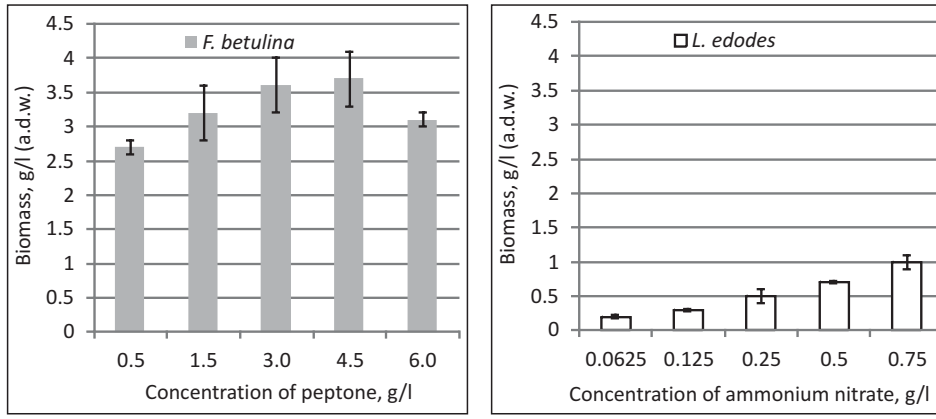

Fig. 7. Effect of nitrogen concentration on *Fomitopsis betulina* (peptone, left) and *Lentinula edodes* (ammonium nitrate, right) biomass growth. Values are presented as means ± standard error of the mean (SEM).

Supplementation of ammonium nitrate enhanced antibacterial activity of *F. betulina* mycelium against *E. coli* and also *B. subtilis*. Nitrogen sources had a significant impact on the antibacterial activity of culture liquids of both fungus species. It is important to note the high activity of *L. edodes* culture liquid against *B. subtilis* regardless of nitrogen source. Moreover, the culture liquid of *L. edodes* was more effective against all tested bacteria than *F. betulina* culture liquid.

Tab. 6. Antibacterial activity of *Lentinula edodes* and *Fomitopsis betulina* for different nitrogen sources. The values represent inhibition zone diameters (mm) and are presented as means ± standard error of the mean (SEM).

Nitrogen source	*Escherichia coli*		*Bacillus subtilis*		*Staphylococcus aureus*	
	Culture liquid	Mycelium	Culture liquid	Mycelium	Culture liquid	Mycelium
Lentinula edodes						
Control*	–	–	–	–	–	–
Asparagine	**FI**	–	**FI**	–	–	–
Urea	15.0 ± 0.0	–	**FI**	–	–	–
Peptone	–	–	**FI**	–	10.7 ± 0.3	–
Ammonium sulphate	–	–	**FI**	–	–	–
Ammonium nitrate	**FI**	–	**FI**	–	10.0 ± 0.5	–
Sodium nitrate	**FI**	–	**FI**	–	–	–
Fomitopsis betulina						
Control*	–	–	–	–	–	–
Asparagine	–	–	11.7 ± 0.3	–	–	–
Urea	–	14.3 ± 1.2	–	–	16.3 ± 0.8	14.5 ± 0.5
Peptone	18.3 ± 1.8	–	15.5 ± 1.6	–	20.8 ± 1.3	–
Ammonium sulphate	–	–	–	–	–	–
Ammonium nitrate	10.5 ± 0.3	13.0 ± 0.4	10.3 ± 0.3	11.0 ± 0.0	12.0 ± 0.0	–
Sodium nitrate	15.5 ± 0.5	–	11.5 ± 0.5	–	–	–

FI = full inhibition of bacterial growth (≥ 25 mm); – = lack of antibacterial activity
* = without nitrogen source

Tab. 7. Effect of ammonium nitrate concentration on antibacterial activity of *Lentinula edodes*. The values represent inhibition zone diameters (mm) and are presented as means ± standard error of the mean (SEM).

Concentration (g/l)	*Escherichia coli*		*Bacillus subtilis*		*Staphylococcus aureus*	
	Culture liquid	Mycelium	Culture liquid	Mycelium	Culture liquid	Mycelium
0.0625	21.7 ± 2.0	–	21.7 ± 1.4	–	22.5 ± 1.7	–
0.1250	**FI**	–	21.6 ± 2.0	–	21.3 ± 1.8	–
0.5000	**FI**	–	**FI**	–	**FI**	–
0.7500	**FI**	–	**FI**	–	**FI**	–
1.0000	**FI**	–	**FI**	–	**FI**	–

FI = full inhibition of bacterial growth (≥ 25 mm); – = lack of antibacterial activity

Tab. 8. Effect of peptone concentration on antibacterial activity of *Fomitopsis betulina*. The values represent inhibition zones (mm) and are presented as means ± standard error of the mean (SEM).

Concentration (g/l)	*Escherichia coli*		*Bacillus subtilis*		*Staphylococcus aureus*	
	Culture liquid	Mycelium	Culture liquid	Mycelium	Culture liquid	Mycelium
0.5	14.7 ± 1.8	–	12.5 ± 0.5	–	14.7 ± 0.9	–
1.5	15.0 ± 0.7	–	18.2 ± 1.7	–	20.0 ± 1.1	–
3.0	15.8 ± 1.2	–	15.4 ± 0.9	–	18.0 ± 1.9	–
4.5	12.5 ± 0.6	–	11.2 ± 0.4	–	11.0 ± 0.6	–
6.0	12.5 ± 0.6	–	11.7 ± 0.9	–	–	–

– = lack of antibacterial activity

Of the tested nitrogen sources, ammonium nitrate and peptone were the most suitable for maximum antibacterial activity of *L. edodes* and *F. betulina*, respectively (Tab. 6). These sources were selected as the most favourable in the following experiments.

The amount of ammonium nitrate was directly proportional to the growth of *L. edodes* (Fig. 7). The presence of ammonium nitrate at concentrations of 0.5–1.0 g/l contributed to the manifestation of antibacterial activity of *L. edodes* culture liquid against all tested bacteria (Tab. 7). An increase of peptone concentration from 1.5 to 4.5 g/l did not cause significantly different *F. betulina* mycelium growth (Fig. 7). On the other hand, addition of peptone at concentrations of 1.5 and 3.0 g/l stimulated the antibacterial activity of *F. betulina* (Tab. 8).

CONCLUSIONS

Successful cultivation of mushrooms (mycelium growth and antibacterial metabolite synthesis) depends on many factors, including the nutritional requirements of the mushrooms. These factors are moreover unique for each fungal species and different fungi strains. The results of our study show the effectiveness of cultivation in liquid static culture for mycelium growth of and antibacterial metabolite synthesis by *Lentinula edodes* and *Fomitopsis betulina*. The studied fungal species showed more or less similar requirements for their growth. Our results indicate that some cultivation conditions (carbon and nitrogen sources, and pH) enhancing mycelial growth are quite different from those necessary for the production of antibacterial substances by the studied mushrooms. Compliance with the established optimum mushroom cultivation conditions will allow for obtaining optimum mycelia amounts with higher antibacterial activity. According to our data, this study is the first report demonstrating the effect of cultivation conditions (carbon and nitrogen sources) on the antibacterial activity of *F. betulina*.

The antibacterial activity of the culture liquid of the fungal species explored in this study was significantly higher than the activity of their mycelia. In the future it will be necessary to identify antibacterial compounds from the liquid culture of *L. edodes* and *F. betulina* and isolate them for further study.

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