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REQUIREMENTS FOR VEGETATIVE GROWTH OF *HOHENBUEHELIA MYXOTRICHA* AND ITS ANTIMYCOTIC ACTIVITY

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Key words: xylotrophic basidiomycetes, *Hohenbuehelia*, macromorphological characters, growth conditions, antimycotic activity.

Abstract

Biometric characteristics of the hyphae and micromorphological features of *Hohenbuehelia myxotricha* mycelia, such as clamps, hyphal loops, crystals were observed by light and electron microscopy. The basic requirements for *H. myxotricha* growth and acquisition of antimycotic activity have been investigated. The highest mycelial growth (18.2 mm/day) of *H. myxotricha* was observed on beer wort agar medium. Suitable conditions for maximal *H. myxotricha* mycelia production were found after 14 days of liquid static cultivation at 25°C, pH 4.5, 30 g/L of glucose, and 2 g/L of yeast extract. The inhibitory effect of *H. myxotricha* was evaluated against *Aspergillus niger*, *Issatchenkia orientalis*, and *Candida albicans* strains. The optimal period for *A. niger* growth inhibition was 14 days while 21 days of *H. myxotricha* cultivation was more appropriated for tested *Saccharomycetales* growth inhibitions. The most suitable for promotion of antimycotic metabolites against all tested pathogenic fungi were glucose and yeast extract.

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Introduction

The cosmopolitan in distribution genus *Hohenbuehelia* Schulzer belonging to Basidiomycota phylum is a relatively small genus with about 40 species known (KIRK et al. 2008). Basic attention has been paid to taxonomically and phylogenetically analysis of *Hohenbuehelia* species based on morphological and molecular data (THORN et al. 2000, KOZIAK et al. 2007, MENTRIDA 2016, CONSIGLIO et al. 2018, LUBIAN et al. 2018, BIJEESH et al. 2019). In contrast, only some cultural characteristics and growth of *H. petaloides* mycelium were investigated *in vitro* (YOO et al. 2001, ZHU et al. 2007). Moreover, *Hohenbuehelia* species have been documented to possess different therapeutical activities. Water and ethanol extracts of *Hohenbuehelia* sp. inhibited the growth of the bacteria *Acinetobacter baumannii*, *Bacillus cereus*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, and fungi *Geotrichum candidum*, *Saccharomyces cerevisiae* (BALA et al. 2012). Compound produced of *Nematoctonus robustus* as known anamorph of *Hohenbuehelia* sp. were active against different pathogenic microorganisms: *A. calcoaceticus*, *Bacillus brevis*, *B. subtilis*, *Candida albicans*, *Micrococcus luteus*, *Mucor miehei* (Current Name: *Rhizomucor miehei* (Cooney & R. Emers.) Schipper), *Nematospora coryli*, *Phodotula glutinis*, *Paecilomyces variotii*, *Penicillium notatum* (Current Name: *Penicillium chrysogenum* Thom), *Saccharomyces cerevisiae* (STADLER et al. 1994). Metabolites from crude extracts of submerged cultures of *H.* grisea exhibited antimicrobial activity against *B. subtilis*, *C. albicans*, *C. tenuis* (Current name: *Yamadazyma tenuis* (Diddens & Lodder) M.A.B. Haase), *M. luteus*, *Mucor plumbeus*, *M. hiemalis*, *Pichia anomala* (Current Name: *Wickerhamomyces anomalus* (E.C. Hansen) Kurtzman, Robnett & Bas.-Powers, *R. glutinis*, *Staphylococcus aureus* (SANDARGO et al. 2018a). Polysaccharides isolated from *Hohenbuehelia serotina* possessed antioxidant (LI et al. 2012, 2017), anti-proliferation (LI et al. 2012, 2017, ZHANG et al. 2014), and can be considered as a radioprotective agent in view of the ability of neutral polysaccharides to significantly improving the activity of glutathione peroxidase (GSH-Px) and increasing the contents of glutathione (GSH) as well as ceruloplasmin in plasma after treated with 6 Gy-radiation (LI et al. 2015). Isolated from *Hohenbuehelia grisea* culture liquids bioactive compounds like 4-hydroxypleurogrisein and pleurotin derivatives (pleurotin, leucopleurotin, dihydropleurotinic acid) displayed antiviral and antimicrobial activities (SANDARGO et al. 2018b). Bioethanol production using *Hohenbuehelia* sp. (strain ZW-16) was also reported (LIANG et al. 2013). Of particular interest is now *Hohenbuehelia* species like *H. grisea* (REALE 2018), *H. portegna*, *H. paraguayensis*,

H. mastrucata (LUBIAN et al. 2018, 2021) due to their potential to be used as biological control of nematodes. One of the rare *Hohenbuehelia* species is *H. myxotricha* which morphology and microanatomy have been studied (ANGELI and SCANDURRA 2012) on the basis of material from Palermo (Sicily). According to taxonomic concept of CONSIGIO et al. (2018) *H. myxotricha* is considered as a synonym of *H. grisea*. Studies of this fungus *in vitro* are very limited. To date, only a few studies were focused on extracellular enzymatic activity (KRUPODOROVA et al. 2014), alternative substrates for its mycelia cultivation (KRUPODOROVA and BARSHTEYN 2015), antimicrobial activity of mycelia and culture liquid (KRUPODOROVA et al. 2016, KRUPODOROVA et al. 2022), and antioxidant potential (KRUPODOROVA et al. 2022). In view of the intensive taxonomic and therapeutic investigations that have been made of *Hohenbuehelia* spp., there is the lack of knowledge in terms of *Hohenbuehelia* spp. morphology, growth and therapeutical activity of mycelium. The aim of current study was to study *H. myxotricha* morphology, growth requirements and antimycotic activity.

Material and Methods

Fungal strains, culture maintenance

Hohenbuehelia myxotricha 1599 was kindly supplied by the Mushroom Culture Collection (IBK) of the M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine (BISKO et al. 2021). Stock cultures were maintained on beer-wort agar-agar slants at 4ºC.

The *Issatchenkia orientalis* Kudryavtsev 301, *Candida albicans* (C.P. Robin) Berkhout 17/138 and clinical strains of *C. albicans* 311, 315, 319 were obtained from the Culture Collection of Microorganisms of the Institute of Epidemiology and Infectious Diseases of the National Academy of Medical Sciences of Ukraine. *Aspergillus niger* Tiegh. IFBG 134 was kindly obtained from the Collection of strains of microorganisms and plant lines of the Institute of Food Biotechnology and Genomics of the National Academy of Sciences of Ukraine. Stock cultures were maintained on potato-dextrose-agar (PDA) slants at 4ºC.

Micro-morphological description

Vegetative mycelium of *H. myxotricha* growing on PDA (the 7th day of growth) was recorded using Zeiss light microscope (MBI-15) and scanning electron microscopy (Jeon JSM – 6060 LA, Japan). A small piece is sepa-

rated from the colony and placing on glass slides in reagent (a drop of sterilized water or Congo red). Preparations for scanning electron microscopy (SEM) were prepared according to QUATTELBAUM and CARNER (1980).

Effect of media on mycelial growth

The effects of different media beer wort agar (WA: liquid beer wort, diluted with distilled water to a density of 8° on the Balling scale, 20.0 g agar), Czapek Dox Agar (CZA, Sigma Aldrich, USA), Malt Extract Agar (MEA, Difco, USA), Potato Dextrose Agar (PDA, Difco, USA), Glucose-Peptone-Yeast Agar (GPYA) 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 10.0 agar on mycelial growth have been examined. Prepared media were autoclaved at 121°C for 20 min. *H. myxotricha* was transferred from stored cultures to PDA Petri dishes and cultured at 26±1°C to obtain mycelial colonies. Mycelial plugs 8-mm were cut from at the mycelial active growth stage using a sterile borer and used as inoculum. Cultivation on solid media was carried out at temperature 26±2 °C in the dark. The mycelia radial growth (RGR, mm/day) was measured daily till the colony covered the full plate and calculated according to the method proposed by WEIS et al. (1999) using the following formula:

$$\mathrm{RGR} = \frac{(R2 - R1)}{(t2 - t1)}$$

where:
RGR – radial growth rate
R – radius of the colony [mm]
t – time [days].

The growth rate (cm/day) was determined according to the formula:

$$\text{Growth rate} = \frac{\text{colony diameter on the last day [cm]}}{\text{number of day's menasurement was taken after inoculation}}$$

Mycelial characteristics on the agar surfaces were recorded. The texture of colonies was performed according to the scale described by STALPERS (1978). The mycelial density was evaluated as follows: very scanty (+), scanty (2+), moderate (3+), abundant (4+), very abundant (5+).

Effect of temperature, pH on mycelial growth

100 ml of liquid glucose-peptone-yeast (GPY) medium in each 250-mL Erlenmeyer flasks was inoculated with three mycelial plugs of 8 mm

diameter cut from the Petri dishes with PDA using a sterile borer in the mycelial active growth stage. To determine the influence of temperature, the inoculated flasks with *H. myxotricha* were incubated at different temperatures (15, 20, 25, 30°C) in incubators for 14 days. To assess the effect of pH level, the inoculated flasks were incubated for 14 days at 25±1°C at different pH levels (4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0). The medium was adjusted to different pH levels used with the addition of 1M NaOH or HCl. After the incubation period, 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.

Effect of incubation time on mycelial growth and antimycotic activity

To study the influence of incubation time, the inoculated 250 mL Erlenmeyer flasks containing 100 mL of GPY nutrient medium and three discs of *H. myxotricha* were incubated at 25°C for different durations (7, 14, 21, 28, 35, and 42 days). After incubation period of cultivation, mycelium was removed, washed with distilled water, dried to constant weight as mentioned above.

To assess the effect of incubation period (14 and 21 days) on antimycotic activity the culture liquid was mixed with previously prepared and cooled to 40°C PDA nutrient medium, in a ratio of 1 : 1. The resulting mixture (a culture liquid with mycelium residues that did not grow on the surface but in the middle of the medium) was poured into Petri dishes with a diameter of 90 mm. After the medium was solidified, one disk of tested pathogenic fungus was introduced into the center of the Petri dish. Control growth of pathogenic fungus was maintained with adding of distilled water to the PDA medium. The observations on colony diameter of each fungus were registrited after the control Petri plates were completely filled with the mycelial growth of the respective fungus. The percent inhibition in mycelial growth was calculated by VINCENT (1947) formula:

$$I = \frac{C-T}{C} \cdot 100$$

where:
I – percent inhibition in colony growth
C – colony growth diameter in control
T – colony growth diameter in treatment.

Effect of carbon and nitrogen sources on mycelial growth and antimycotic activity

To assess the influence of carbon and nitrogen sources was chosen glucose-asparagine medium consisted of (g/L): 10.0 glucose, 0.4 asparagine, 1.0 KH_2PO_4, 0.5 $MgSO_4 \cdot 7H_2O$ (Bisko and Kosman 1988). Monosaccharides (arabinose, dextrose, galactose, glucose, fructose, xylose), disaccharides (lactose, maltose, sucrose), polysaccharide (soluble starch) and mannitol were used as carbon sources. For each of them, 4 g/L of C (pure carbon per liter calculated as the percentage of the carbon element in a molecule) were added individually to the medium to replace glucose. Different amounts of glucose (10–55 g were dissolved in 1000 mL of the medium) were used to assess the most suitable for mycelial growth carbon source concentration.

Different nitrogen sources (ammonium nitrate, ammonium sulphate, L-asparagine, peptone, sodium nitrate, yeast extracts, and urea) were used for the study. For each of them, 0.21 g/L of N (pure nitrogen per liter calculated as the percentage of nitrogen element in a molecule) was added individually to the medium to replace asparagine. Different amounts of yeast extracts (0.5–2.5 g were dissolved in 1000 mL of the medium) were used to assess the most suitable for mycelial growth nitrogen source concentration. 100 mL of medium in 250-ml Erlenmeyer flasks was inoculated with three mycelial plugs (8 mm diameter) of *H. myxotricha* and was incubated at 25°C for 7 days. After the incubation period, mycelial growth as well as antimycotic 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 analyzed 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

The use of fungi is very actually and perspective trend due to their wide range of applications in different modern areas. The cultural and morphological properties of the vegetative mycelium of fungi are one of the integral and important parts of a comprehensive study of species in cul-

ture that can be used as additional criteria for solving taxonomic and biotechnological purposes (BUCHALO et al. 2011, BADALYAN et al. 2015, MYKCHAYLOVA et al. 2019, 2021). The determination of the optimal medium is the simplest and most essential tool for the introduction and as well as for improving the cultivation of fungi in culture.

Used media had a strong effect on colony morphology (Figure 1, Table 1) and growth of studied fungus (Table 1). The radial growth, average growth rate, texture, color, form, surface, margin, density of the culture mat, and its reverse color were taken into account as diagnostic macromorphological characteristics of *H. myxotricha* cultivated on various media (Table 1). *H. myxotricha* displayed a significant growth in all media used. However, the WA medium was the most suitable for mycelial growth of *H. myxotricha*.

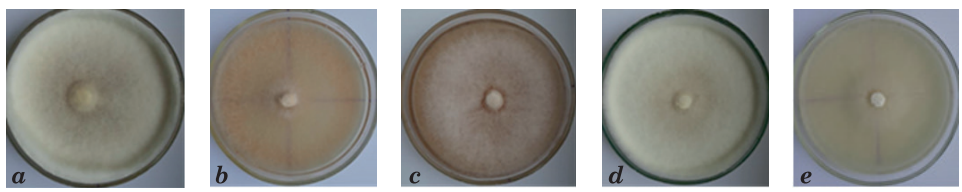

Fig. 1. Mycelial colonies of *H. myxotricha* on 9 days of cultivation on different media: *a* – WA – beer wort agar; *b* – PDA – potato dextrose agar; *c* – MEA – malt extract agar; *d* – GPYA – glucose-peptone-yeast agar; *e* – CZA – Czapek dox agar

Table 1

Colony morphology and growth of *H. myxotricha* cultivated on various media

Medium	Mycelial characteristics					Growth		
	texture	color	form/surface/ margin	density	reverse	mycelial growth	radial growth, [mm/day]	growth rate, [cm/day]
WA	felty	off-white	circular/ flat/entire	5+	unchanged	abundant	18.2±0.5	2.2±0.1
PDA	downy	white	circular/ flat/entire	3+	light brown	regular	10.7±0.3	1.5±0.1
MEA	downy	white	circular/ raised/entire	4+	unchanged	regular	8.8±0.4	1.3±0.2
GPYA	felty	off-white	circular/ flat/entire	5+	unchanged	abundant	12.2±0.2	1.8±0.1
CZA	silk thread, quickly aggregating	white	circular/ flat/entire	+	unchanged	scarce	11.2±0.1	1.5±0.0

Media: WA – beer wort agar; PDA – potato dextrose agar; MEA – malt extract agar; GPYA – glucose-peptone-yeast agar; CZA – Czapek dox agar. The mycelial density: very scanty (+); scanty (2+); moderate (3+); abundant (4+); very abundant (5+)

Biometric characteristics of the hyphae, clamp of *H. myxotricha* and crystals shown in Figure 2. The mycelium is unstained, contains inclusions of fat. Vegetative mycelia are septated, branched, of different thickness from 2.5 µm to 12.7 µm, anastomosed, with conspicuous clamp-connections (giant, single, pairs, whorls, oval, almond-shared), irregular hyphal loops (mycelial rings or trap) and crystals. Calcium oxalate crystal formation is quite a widespread phenomenon of wood-rotting basidiomycetes that may potential importance in the oxalate carbonate pathway (GUGGIARI et al. 2011). Therefore, several aspects of the microscopic characteristics of *H. myxotricha* mycelium can be useful in distinguishing between *Hohenbuehelia* taxa as well as in quality control of mycelial cultures by fungi cultivation for biotechnological purposes.

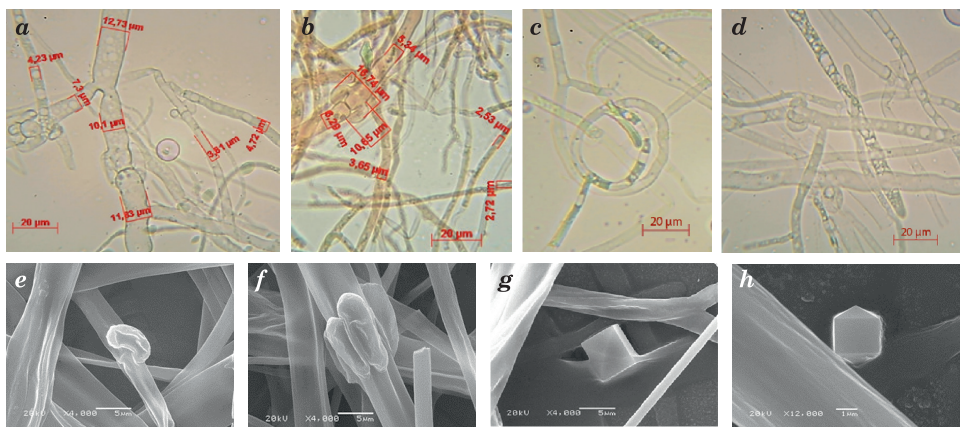

Fig. 2. Hyphal morphology of *H. myxotricha*: *a*, *b* – biometric characteristics of the hyphae; *c* – hyphal loops; *d–f* – hyphal growth of mycelium and clamp-connections; *g*, *h* – crystals. Bar = 20 µm (*a–d*) light microscopy; 5 µm (*e–g*) and 1 µm (*h*) scanning electron microscopy

The growth conditions can have significant impacts on the biomass and valuable metabolite production. And the determination a suitable fungi growth temperature is an important starting point for its cultivation *in vitro*. Basidiomycetes can grow in a wide range of temperatures, and *H. myxotricha* grew at all temperatures used. Our temperature investigations shown that *H. myxotricha* is a mesophilic fungus. The best mycelial growth (10.2 ±0.1 g/L) was observed at 25°C (Figure 3). This temperature was optimal for *Hohenbuehelia petaloides*, strain Hp 831 (ZHU et al. 2007). However, for other strain KACC 500040 of *H. petaloides* maximal mycelial growth was found at 30°C (YOO et al. 2001).

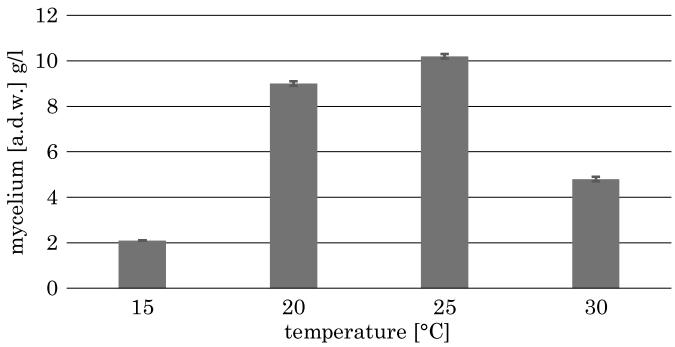

Fig. 3. Effect of temperature on mycelial growth of *H. myxotricha*

It is known that fungi have multiple mechanisms which allows them to adapt to pH changes. *H. myxotricha* grown in a wide range of initial pH levels (Figure 4). Suitable initial pH level of 4.5 provided maximal mycelial growth of this fungus at 11.6 ± 0.0 g/L. For mycelial growth of *H. petaloides* established other optimal pH levels: 6.0 (YOO et al. 2001), and 6.0–7.0 (ZHU et al. 2007).

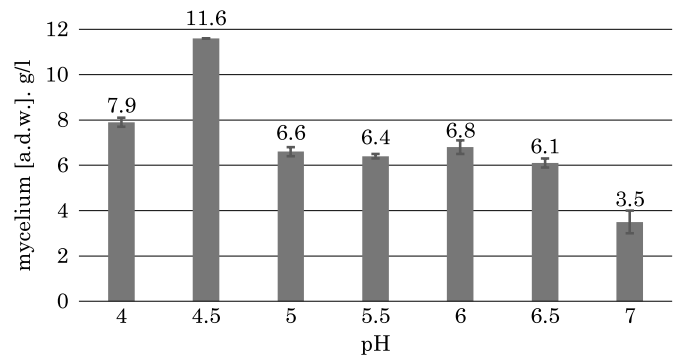

Fig. 4. Effect of pH on mycelial growth of *H. myxotricha*

The conditions for the correct time incubation are key to further understanding the dynamics of biomass production. The incubation period of 14 days was optimal for the best mycelial growth (9.4±0.3 g/L) of *H. myxotricha* (Figure 5). It should be noted that pH of cultivation medium was at constant level 4.0 starting up 7 to 42 days. This tendency points that fungus can change environment pH level close to its optimal parameter (Figure 4). It is in line with the capacity to control extracellular pH level that is an important attitude of fungal physiology.

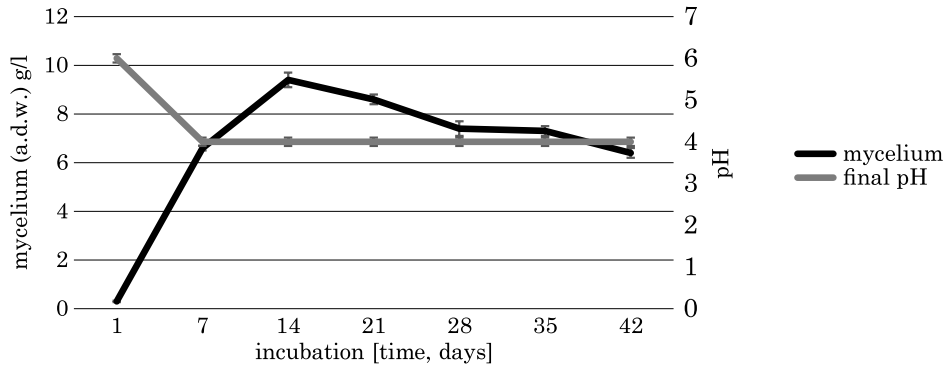

Fig. 5. Effect of incubation time on mycelial growth of *H. myxotricha*

It is well known that carbon sources are one of the most important nutrients required for the growth of living microorganisms. The presence of strong fermentative complex of *H. myxotricha* provided this fungus to assimilated all tested carbon sources at different level (Figure 6).

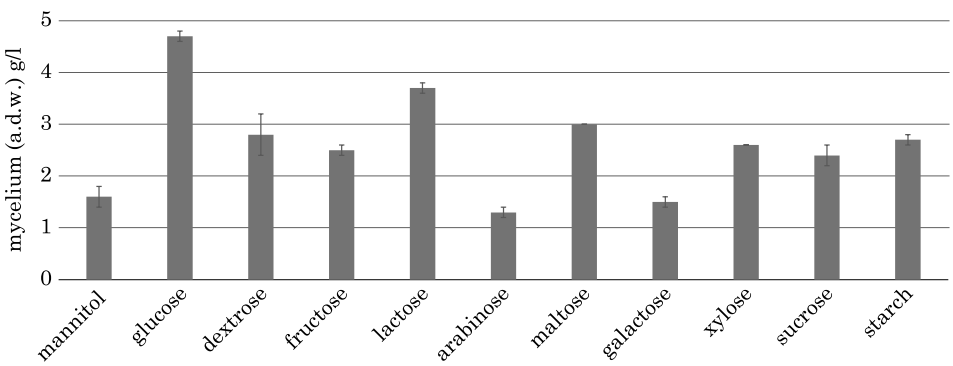

Fig. 6. Effect of carbon sources on mycelial growth of *H. myxotricha*

Among the eleven C-sources, glucose supported the best mycelial growth (4.7±0.1 g/L) of *H. myxotricha*, followed by lactose (3.7±0.1 g/L). The optimal concentration of glucose was 30 g/L, since we did not observe a statistically significant difference by further its increasing in the nutritional medium (Figure 7). Stimulatory effect of glucose may be explained that it is the main respiratory substrate and due to the ease with which this monosaccharide can metabolized to produce cellular energy (PAPASPYRIDI et al. 2012). While arabinose, galactose and mannitol were the most unfavorable C-sources. Dextrine (in concentration of 10%), fructose (in concentration of 10%) and lactose stimulated greater mycelial growth of *H. petaloides* (Yoo et al. 2001). Sodium carboxymethylcellulose was the most favorable for the growth of other strain of *H. petaloides* (ZHU et al. 2007).

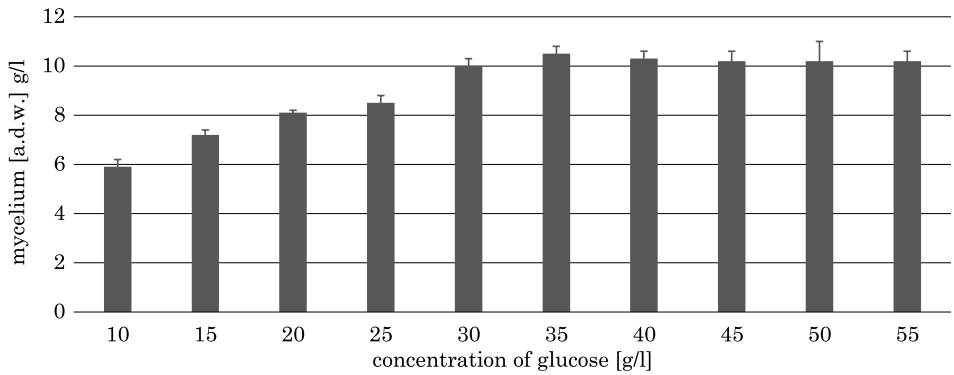

Fig. 7. Effect of glucose concentration on mycelial growth of *H. myxotricha*

The nitrogen sources have been shown to be essential to attitudes of fungi development and growth. All the six nitrogen sources tested were found suitable for investigated fungus growth (Figure 8). However, its capability to utilize various forms of nitrogen was differs. The most suitable nitrogen was yeast extracts that provided the highest mycelial yield 4.3±0.3 g/L.

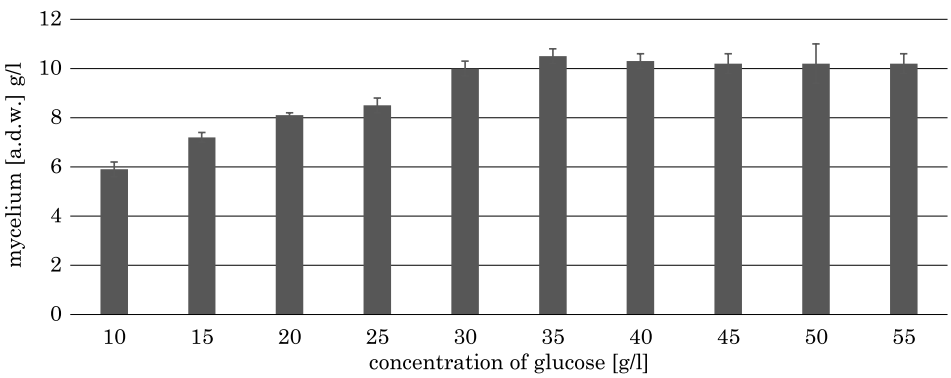

Fig. 8. Effect of nitrogen sources on mycelial growth of *H. myxotricha*

This effectivity of yeast extract can be explained due to its complexity composition like amino acids, carbohydrates, proteins, minerals, and vitamins. Yeast extract the best enhanced mycelial growth of *H. myxotricha* at concentration of 2 g/L (Figure 9). General, organic nitrogen sources were more suitable for *H. myxotricha* growth then inorganic N-sources. Tryptone, soytone, malt extract and also yeas extract (at concentration of 0.4%) were favorable for mycelial growth of *H. petaloides* (YOO et al. 2001). While peptone was the major nitrogen sources that supported the best mycelial growth of other strain of *H. petaloides* (ZHU et al. 2007).

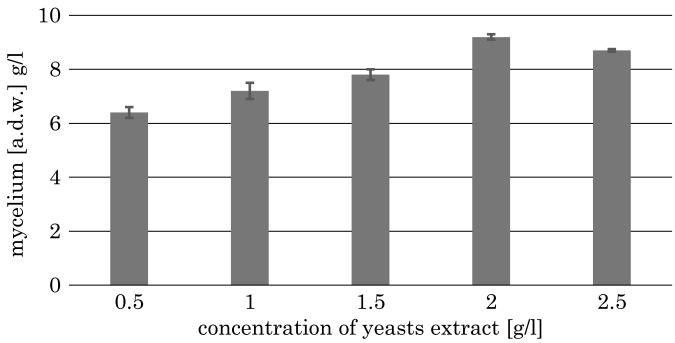

Fig. 9. Effect of yeast extract concentrations on mycelial growth of *H. myxotricha*

Fungal metabolites contributed the development of active substances in the modern pharmaceutical industry as well as promising agents to improve human and animal health. Our previously results showed that the ethanolic extract of *H. myxotricha* mycelia possessed strong antimicrobial potential, particularly antifungal activity (KRUPODOROVA et al. 2022). Metabolite's productions depend on different factors that vary among species, and also strains. The variation of growing condition can lead to significantly increased formation of a metabolites of interest. Incubation period affected the display of antimycotic activity of *H. myxotricha* (Figure 10).

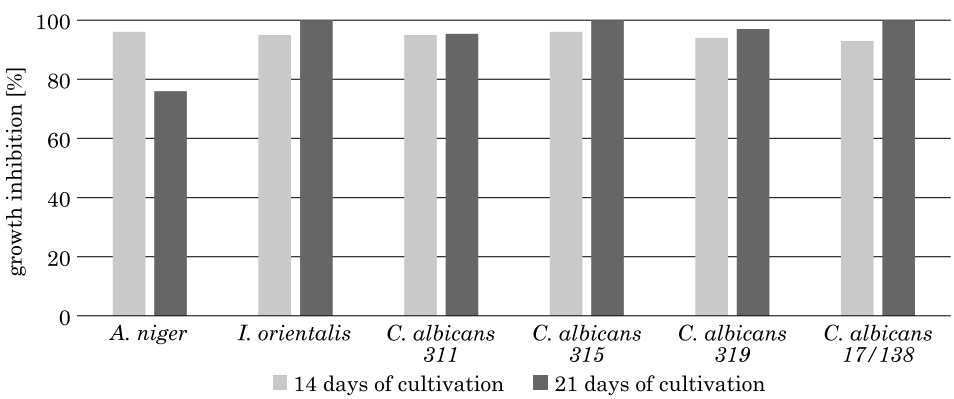

Fig. 10. Effect of incubation periods on antimycotic activity of *H. myxotricha*

It could be found that incubation time have in impact on the production on antimycotic metabolites of *H. myxotricha*. After 14 days of cultivation the antimycotic activity of *H. myxotricha* was significantly more effective against *A. niger* compared to other cultivation period. Increasing of incubation period to 21 days possessed maximal growth inhibition of *I. orientalis*, *C. albicans* 315, 319, 17/138. Suppression of *C. albicans* 311

growth was established at the same level (95%) regardless of the duration *H. myxotricha* cultivation. Mention above observation is in line with other studies (DYAKOV et al. 2011, SAZANOVA et al. 2013, BARAKAT and SADIK 2014, POPOVA 2015, SILVA et al. 2016, KRUPODOROVA et al. 2022) which reported that antimicrobial activity of Basidiomycetes (*Cantharellus cibarius* Fr., *Flammulina velutipes* (Curtis) Singer, *Fomitopsis betulina* (Bull.) B.K. Cui, *Lentinula edodes* (Berk.) Pegler, *Lentinus tigrinus* (Bull.) Fr., *Pleurotus ostreatus* (Jacq.) P. Kumm., *P. pulmonarius* (Fr.) Quél., *Pholiota lenta* (Pers.) Singer, *Rhodocollybia maculata* (Alb. & Schwein.) Singer) can varied depending on the duration of their cultivation time at different stage of their development.

Variation of nutritional conditions influenced also the production of antifungal secondary metabolites of *H. myxotricha* (Figure 11, Figure 12).

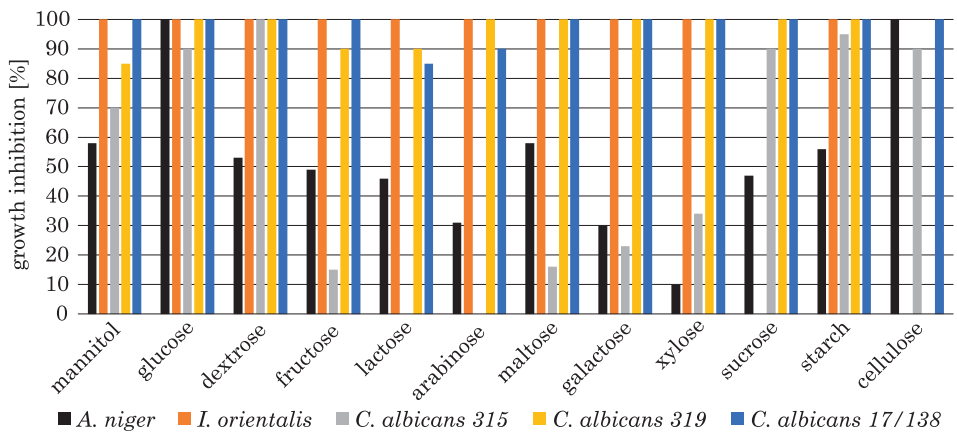

Fig. 11. Effect of carbon sources on antimycotic activity of *H. myxotricha*

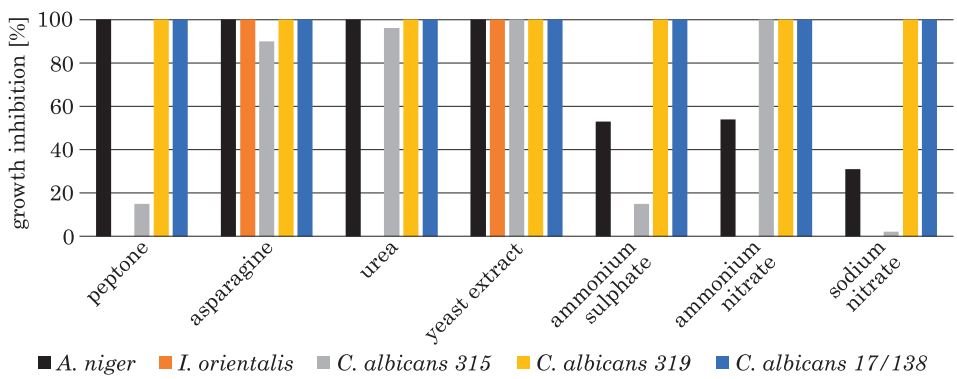

Fig. 12. Effect of nitrogen sources on antimycotic activity of *H. myxotricha*

The activity significantly varied depend of used carbon sources, the inhibition ranged from 10 to 100 % (Figure 11). It should be noted that we didn't observed activity against *C. albicans* 319 in case of cellulose application, against C. *albicans* 315 by present in the medium lactose or arabinose, and against *I. orientalis* by using of cellulose and sucrose. The most suitable for antimycotic metabolites promotion was glucose. Starch and dextrose inhibited growth of pathogenic fungi also good. The revealed need for the selection of optimal carbon sources to increase the antimicrobial activity is in agreement with previous reports (BARAKAT and SADIK 2014, POPOVA 2015, SILVA et al. 2016). Maltose was optimal for suppression of *A. niger* growth in experiment with *Pleurotus ostreatus* (BARAKAT and SADIK 2014). Growth inhibition of *Candida* spp. supported the presence in culture medium glucose and fructose in case of cultivation *P. ostreatus* (BARAKAT and SADIK 2014) and *Cantharellus cibarius* (POPOVA 2015), respectively. While glucose and starch by *P. pulmonarius* cultivation were suitable carbon sources for inhibition of *C. albicans* growth (SILVA et al. 2016).

The different effect of nitrogen sources on antimycotic activity of *H. myxotricha* was found (Figure 12). The activity greatly varied depending on the presence of nitrogen sources and the inhibition ranged from 2 to 100%, and similar dependence was also found previously for *Pleurotus ostreatus* submerged cultivation (VAMANU 2012). The most demanding under the conditions of the nitrogen experiment was *I. orientalis*. However, the most antimycotic activity of studied fungus against all used fungi was stimulated by yeast extract. Ammonium nitrate also good promoted growth suppression of all *C. albicans* strains. In general, organic nitrogen sources were more appropriated for antimycotic activity of *H. myxotricha* then inorganic N-sources. Ammonium sulfate, resulted in obtaining a mycelium *P. ostreatus* has shown the most pronounced antimicrobial effect (VAMANU 2012).

Conclusion

The results of our study demonstrated that *Hohenbuehelia myxotricha* can be successful growth at different conditions in culture. This is the first report concerting to detailed information about the cultural and morphological properties of the vegetative mycelium of *H. myxotricha* and on effects of basic nutritional requirements for its growth and some metabolites production. The growth conditions can have significant impacts on the colony morphology, mycelial growth and antimycotic compounds production. Additionally, abilities to *H. myxotricha* grow at different media,

pH levels, temperatures, as well as the capabilities of using wide ranges of C- and N- sources allow this fungus to adapt to changing conditions. This study has shown that maximal mycelia of *H. myxotricha* can be established after 14 days of liquid static cultivation at 25°C, pH 4.5, glucose at 30 g/L concertation, yeast extract at 2 g/L concentration. The ability to increase and ensure a controlled level of antimycotic activity of *H. myxotricha* indicates the viability of biotechnological and pharmaceutical applications of this species of fungus. Incubation period and nutritional needs affected the display of antimycotic activity of *H. myxotricha*. The optimal period for growth inhibition of *Aspergilus niger* was 14 days while 21 days of cultivation was more appropriated for growth inhibitions of *Saccharomycetales* used. The most suitable for promotion of antimycotic metabolites were glucose and yeast extract. In general, obtained results add to our knowledge of the environmental features of studied fungus and its secondary metabolites for axenic cultivation. Further work is needed to isolation and identification of the active compounds responsible for antimycotic activity that can provide new potential perspective compounds for modern pharmacology.

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Competing Interests

The authors declare that they have no competing interests.

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