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Evaluation of *Fomitopsis betulina* strains for growth on different media and exopolysaccharide production

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Abstract: *Fomitopsis betulina* is a widespread macromycete with valuable medicinal potential. This study screened 22 different *F. betulina* strains for mycelial growth on various media and exopolysaccharide production. Strain-specific features of *F. betulina* growth and exopolysaccharide production on different media were observed. Variations in the growth rate of the studied strains ranged from 3.50±0.33 to 8.75±0.50 mm/day, biomass synthesis from 2.28±0.26 to 13.72±0.05 g/L, and exopolysaccharide production from 0.02±0.00 to 2.20±0.31 g/L. Maltose as a carbon source in malt extract agar (MEA) and malt extract broth (MEB) was the most suitable for the growth, while dextrose and starch as carbon sources in potato dextrose broth (PDB) were more suitable for exopolysaccharide production. The *F. betulina* 311 strain has significant biotechnological potential, demonstrated by its robust growth on different agar media, efficient biomass synthesis, and high production of extracellular biopolymers. Our results highlight the significance of different growth media and fungal strains in optimizing biomass and exopolysaccharide production.

Keywords: Birch polypore, biomass, media, mycelium, exopolysaccharide

INTRODUCTION

Fungi cultivation is one of the most economically important industrial and biotechnological sectors that has expanded significantly worldwide. Among brown rot basidiomycetes, the polypore fungus *Fomitopsis betulina* (Bull.) B. K. Cui, M. L. Han & Y. C. Dai (birch polypore) has been used in folk medicine for years [1–6]. Since ancient times, its fruit body powder has been well-known in different countries for its antiseptic, antimicrobial, blood clotting, and painkiller properties [1,2,7,8]. *F. betulina* has antiparasitic and immunoenhancing applications and is used for treating stomach disorders [6].

The fruiting bodies of *F. betulina* contain bioactive substances with valuable therapeutic properties. Most attention has been paid to studying its anticancer [1,9–14] and antimicrobial [15–21] activities. Additionally, research has focused on its anti-inflammatory properties [6,11]. Some activities, such as antioxidant [22], wound-healing [23], and immune-enhancing [24], have been studied fragmentarily. The biological activity of this polypore fungus is mainly attributed to triterpenoids [6,12,16,25]. Scientists have also focused on polyporenic acids [6,12,13,21] and phenolic compounds [11]. Despite polysaccharides being one of the most important compounds responsible for the therapeutic activities of mushrooms, glucans have been studied only sporadically [22,23,26].

Most studies have been conducted on the fruiting bodies of *F. betulina*, with fewer focusing on its mycelium and liquid culture. Some studies have been devoted to *F. betulina* mycelial growth: morphological and cultural properties of *F. betulina* on agar media [27,28], the influence of some basic cultivation conditions and ultraviolet C irradiation on *F. betulina* growth [19,20], and the effect of different carbon

and nitrogen sources, as well as medium pH, on the growth of 11 *F. betulina* strains [29].

Currently, *F. betulina* is the subject of research by mycologists, biotechnologists, and pharmacologists due to its established medicinal properties. Research with pure cultures not only deepens the understanding of their nutritional, chemical, and environmental requirements for growth and metabolism but also facilitates the discovery and identification of new compounds and activities. The practical implementation of mycobiotechnologies requires a scientifically based selection of promising producer strains and expanded knowledge about their growth and metabolite synthesis. Since metabolites are produced in varying amounts depending on the culture conditions, studying *in vitro* culture conditions to optimize their production is relevant and of great practical importance. However, limited information on the stimulatory effects of the culture medium on the growth and metabolite production of *F. betulina* has been reported in the literature. It is important to note that the growth of mycelium and the synthesis of metabolites significantly depend on the strain of the fungus [30-32]. Therefore, this study aimed to screen 22 different *F. betulina* strains for mycelial growth on various media and exopolysaccharide production.

MATERIALS AND METHODS

Ethics statement

This research does not involve any ethical issues.

Nomenclature

The fungus used in the present study was *Fomitopsis betulina* (Bull.) B. K. Cui, M. L. Han & Y. C. Dai (birch polypore), a member of the Fomitopsidaceae family.

Fungal cultures

Twenty-two *F. betulina* strains were examined in this study. All strains (designated *F. betulina* 311, 327, 978, 988, 989, 2269, 2290, 2363, 2364, 2366, 2399, 2770, 2771, 2772, 2773, 2774, 2775, 2776, 2777, 2778, 2785, and 2786) were generously provided by the Mushroom Culture Collection of the M.G. Kholodny Institute of Botany (IBK) of the National Academy of Sciences of Ukraine [33]. All cultures were maintained on the malt extract agar (MEA, Thermo Fisher Scientific, USA) slants at 4°C.

Culture media and cultivation conditions

Potato dextrose agar (PDA; Difco, USA), Sabouraud dextrose agar (SDA; HiMedia Pvt Limited, Mumbai, India), MEA, and glucose peptone yeast agar (GPYA) contained g/L: 25 g glucose (Lycadex® PF, France), 3 g yeast extract (Conda, Spain), 2 g peptone (Conda, Spain), 1 g K_2HPO_4 (Hebei Ruisite Technology Co., Ltd, China), 1 g KH_2PO_4 (Haifa Group, Israel), 0.25 g $MgSO_4$ x7H_2O (Poluks Group, Poland), and 20 g agar (Conda, Spain). The following 4 media without agar: potato dextrose broth (PDB), malt extract broth (MEB), Sabouraud dextrose broth (SDB), and glucose peptone yeast broth (GPYB) were used as liquid media for surface liquid static cultivation. All prepared media were autoclaved for 15 min at 121°C. The mycelium of each *F. betulina* strain was transferred from slants to GPYA Petri dishes and then incubated in the dark at 25°C with ventilation for 10 days. Inoculum (1 mycelial disk 7 mm in diameter from 10-day cultures of *F. betulina* strains) was added to the plates containing a specific medium and incubated at 25°C until the medium in the Petri dish was completely covered with the strain mycelium. Inocula (3 mycelial disks 7 mm in diameter from 10-day cultures of *F. betulina* strains) were added to the flasks with a specific medium and incubated at 25°C for 14 days.

Growth and biomass assay

The growth of fungal strains on agar media was measured by the radial growth rate (RGR, mm/day) according to the following equation [34]:

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

where RGR is the radial growth rate, R2 is the colony radius at the end of the linear growth phase in mm, R1 is the colony radius at the beginning of the linear growth phase in mm, and t2-t1 is the duration of the linear growth phase in days.

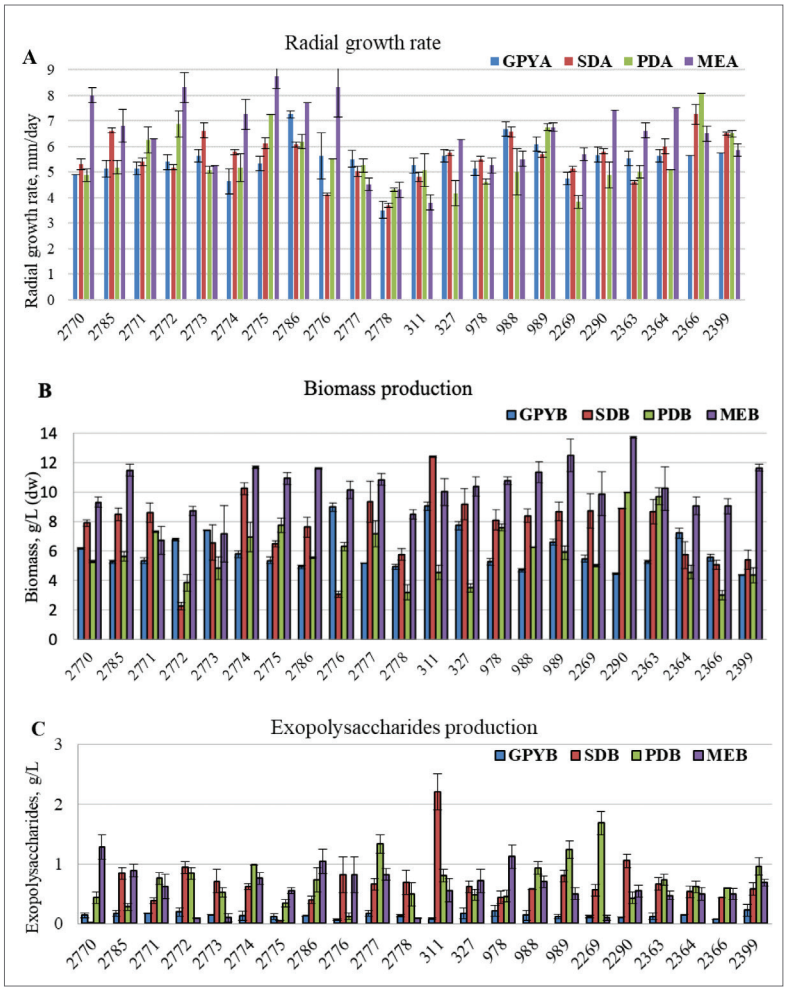

Fig. 1. Effect of media on Fomitopsis betulina strains: **A** – radial growth rate, **B** – biomass production, **C** – exopolysaccharide production. GPYA – glucose peptone yeast agar, SDA – Sabouraud dextrose agar, PDA – potato dextrose agar, MEA – malt extract agar, GPYB – glucose peptone yeast broth, SDB – Sabouraud dextrose broth, PDB – potato dextrose broth, MEB – malt extract broth. Data are expressed as the mean±SD (n=3).

The growth of fungal strains in liquid media was quantified by measuring biomass and expressed as gram dry weight (dw) per liter (g/L). Mycelia grown in liquid medium were separated from the medium by filtration using Whatman N 4 filter paper, washed twice with distilled water, and dried at 85°C to constant weight.

Exopolysaccharides assay

After biomass separation, the culture broth was evaluated on exopolysaccharide (EP) production. EPs were obtained by precipitation with 95% ethanol, applied in a 1:4 (v/v) ratio of supernatant to ethanol. After an overnight at 4°C, the mixture was separated by centrifugation at 7,245×g for 10 min. The precipitate was then collected, dialyzed against water, and dried at 60°C. The crude EP content in the precipitate was examined by the phenol-sulfuric acid method [35], with glucose used as a reference.

Statistical analysis

All experiments were performed three times for each variant. Values were expressed as the means±standard deviation. An analysis of variance (ANOVA) was performed to test for significance between variables using one-way ANOVA, calculated with Statistica 11.5 (StatSoft Inc., USA), followed by Tukey's HSD test. Differences were considered significant at $P<0.05$.

RESULTS

The study screened 22 different *F. betulina* strains for mycelial growth on various media and EP production.

Growth on agar media

The influence of each medium on the growth of *F. betulina* strains was established, with the growth rate varying from 3.50±0.33 to 8.75±0.50 mm/day (Fig. 1A). GPYA supported the maximal growth rate of *F. betulina* 2786 (7.25±0.12 mm/day). SDA and PDA promoted the best growth of *F. betulina* 2366 (7.25±0.40 and 8.06±0.00 mm/day, respectively), while MEA was best for *F. betulina* 2775 growth (8.75±0.50 mm/day). The lowest growth rates on all tested media were observed in only one strain – *F. betulina* 2778. One particular medium proved more suitable for the growth of most strains (59.09%). Specifically, 11 strains (50%) showed optimal growth on MEA, *F. betulina* 2773 thrived on SDA, and *F. betulina* 2366 exhibited the best growth on PDA. Some strains (27.27%) were able to grow at the same rate on different media: on SDA and MEA – *F. betulina* 2785,

on PDA and MEA – *F. betulina* 989, 2771, 2778, on PDA and SDA – *F. betulina* 2399, on GPYA and SDA –*F. betulina* 989. It should be noted that some strains showed equal growth rates on three tested media: *F. betulina* 311 and 2777 on GPYA, SDA, and PDA, and *F. betulina* 978 on GPYA, SDA, and MEA.

Growth on liquid media

All tested liquid media were suitable for biomass production of *F. betulina* strains (Fig. 1B). The amount of obtained mycelial biomass significantly varied from 2.28±0.26 to 13.72±0.05 g/L depending on the media and used strain after 14 days of growth. GPYB and SDB media supported maximum mycelium biomass for *F. betulina* 311 (9.06±0.01 and 12.44±0.06 g/L, respectively); PDB and MEB provided maximum growth of *F. betulina* 2290 (9.98±0.02 and 13.72±0.05 g/L, respectively). Of the 4 tested media, MEB was the best medium for biomass production for most studied strains (72.73%) whereas SDB was favorable for strains 311 and 2771. Certain strains were adapted to grow in different media producing the same amount of biomass: strains 327 and 2269 in MEB and SDB; growth: strain 2363 in SDB, PDB, MEB; cultivation: strain 2773 in GPDB, SDB, MEB.

EP production

Our results indicated that studied *F. betulina* strains were capable of producing EPs when grown in all used media (Fig. 1C). EP formation varied from 0.02±0.00 to 2.20±0.31 g/L depending on the culture medium and the strain used on day 14 of growth. GPYB was the most suitable medium for achieving maximum EP production in *F. betulina* 2399 (0.23±0.10 g/L). For *F. betulina* 311, SDB was optimal (2.20±0.31 g/L). PDB and MEB yielded the highest EP formation in *F. betulina* 2269 (1.68±0.40 g/L) and 2770 (1.28±0.41 g/L), respectively. Of the 4 tested media, the lowest EP content was observed during cultivation in GPDB. One particular medium proved more favorable for EP production in a majority of strains (54.54%): MEB was effective for strains 978, 2770, and 2775; PDB for strains 988, 989, 2269, 2366, 2399, 2774, and 2777; and SDB for strains 311 and 2290. Some strains (36.36%) were capable of producing the same EP content in different media. This trend was observed for strains 2363, 2772, 2773, and 2778 in SDB and PDB; for strains 2776 and 2785 in SDB and MEB; for strains 2771 and 2786 in PDB and MEB. EP synthesis in *F. betulina* 327 and 2364 did not show statistically significant differences across three cultural media (SDB, PDB, and MEB).

We investigated the potential correlation between biomass and EP production of the studied strains grown in different media. Pearson's correlation coefficient values are presented in Table 1. Our results showed a positive correlation between biomass and EPs in 2 media, SDB and MEB. This correlation is weak (R=0.32 and 0.38) according to the scale proposed by Evans [36].

Table 1. Pearson's correlation coefficient (R) between fungal biomass and EP production in studied *Fomitopsis betulina* strains

Biomass				
Media	**GPYB**	**SDB**	**PDB**	**MEB**
EPs	-0.27754	0.324725	-0.05111	0.381848

GPYB – glucose peptone yeast broth, SDB – Sabouraud dextrose broth, PDB – potato dextrose broth, MEB – malt extract broth, EPs – exopolysaccharides.

Variations in the growth rate, biomass synthesis, and EP production of the studied strains have been established. Agar and liquid media based on malt extract provided the maximum growth effect for most strains, while liquid potato dextrose medium was more suitable for EP production. The *F. betulina* 311 strain has biotechnological potential attributed to its growth ability on different agar media, biomass synthesis capability, and highest EP production.

DISCUSSION

There is a growing interest in studying *F. betulina* fruiting bodies to find a new source of valuable biologically active metabolites with a wide range of beneficial effects. However, there needs to be more information in the scientific literature about the growth and physiological characteristics of *F. betulina* strains. This work focuses on the strain-specific features of *F. betulina* growth in different media and EP production. Our results illustrate the diverse growth capabilities of the studied *F. betulina* strains on the media used. The growth rate is a crucial characteristic of fungi that should be considered when evaluating the biotechnological potential of a culture. The growth characteristics of mycelia can be determined as the

mycelial growth rate (GR) and the average growth rate (GR_{avr}) [37-39] or as radial growth rate (RGR or Vr) [34,40,41]. The 22 strains of *F. betulina* examined were capable of growth, with some variations depending on the agar media used: RGR=3.50-7.25 mm/day (GPYA), RGR=3.69-7.25 mm/day (SDA), RGR=3.83-8.06 mm/day (PDA), RGR=3.80-8.75 mm/day (MEA). Slightly better results were observed when the growth characteristics of 11 strains of *F. betulina* were studied [33]. Their growth was recorded on 4 agar media as follows: Vr=4.1-7.2 mm/day (GPYA), Vr=4.6-7.5 mm/day (beer agar), Vr=5.0-9.5 mm/day (PDA), Vr=4.8-7.5 mm/day (MEA). It was established that the average growth rate (GR_{avr}) for the three studied strains of *F. betulina* was 3.6-4.9 mm/day on beer agar [28]. Based on proposed growth strategies [42], all studied *F. betulina* strains belong to the intermediate P-strategist group, with a growth rate (Vr) ranging from 1.1 to 11 mm/day. Overall, the growth rate of the studied strains remained consistent, with only *F. betulina* 2778 exhibiting the lowest relative growth rate (RGR) on the 4 agar media. The culture medium is also a critical factor affecting mycelial growth *in vitro*, and screening is to some extent the starting point for determining the biosynthetic potential of a culture. We tested standard media used for basidiomycete cultivation, including MEA and PDA [43]. GPYA media were also investigated in a previous study on the growth of *F. betulina* strains [27]. Based on growth measurements in this study, the agar media can be ranked as follows: MEA>SDA>PDA>GPYA. However, the PDA medium was favorable for most studied *F. betulina* strains in other similar investigations [27]. In our study, the preference for a particular medium for biomass synthesis corresponds to the same order as the growth of *F. betulina* strains on the agar media used. Analysis of biomass production indicates that the highest growth after two weeks, depending on the optimal liquid medium, can be ranked as follows: MEB>SDB>PDB≥GPYB. From our results, maltose as a carbon source in MEA and MEB was the most suitable for the growth of most of the studied *F. betulina* strains on the tested media. All studied media, except for PDA and PDB, contained peptone as a nitrogen source (in varying amounts from 2 to 10 g). GPYA and GPYB media also included yeast extract (3 g) as a nitrogen source. The suitability of the culture media used for the growth of *F. betulina* strains is also determined by the varying ratios of carbon and nitrogen sources required by each strain. Overall, agar and liquid media containing malt extract facilitated maximum growth owing to their rich and complex natural composition. The differences in growth of the studied strains, depending on the media used, are due to the availability of accessible nutrients in the medium, including inorganic or organic compounds, carbohydrates, nitrogen, and their ratios, as well as the nutritional requirements of each strain.

The 22 strains of *F. betulina* showed significant differences in mycelial biomass production depending on the liquid media used: 4.40-9.06 g/L (GPYB), 2.28-12.44 g/L (SDB), 3.03-9.98 g/L (PDB), 6.75-13.72 g/L (MEB). According to these data, using maltose as a carbon source in the nutrient medium tends to result in a greater yield of mycelial biomass. MEA and Hagem medium, both containing malt extract, were utilized for growing *F. betulina* [17]. The biomass production of the studied *F. betulina* strains was higher than other *F. betulina* strains grown on a synthetic medium: 0.9-4.7 g/L (depending on tested carbon sources) and 1.1-4.7 g/L, depending on tested nitrogen sources [29]. We obtained a slightly lower amount of synthesized biomass (7.74 g/L) of *F. betulina* strain 327 on GPYB compared to our previous study [19], where we observed 8.1 g/L biomass on the same medium. UV irradiation of *F. betulina* strain 327 reduced biomass production (2.6-3.7 g/L) when grown in GPYB medium [20]. *F. betulina* can also be cultivated on Oddoux medium [11].

Another important biotechnological characteristic of any culture is the production of bioactive compounds during growth. Fungal EPs are significant extracellular metabolites that evolutionarily play a crucial role in adaptation and functionality [44,45]. In recent years, considerable attention has been paid to EPs, recognized as highly valuable compounds with numerous applications [46-48]. We focused our attention on studying the levels of EP production. To our knowledge, this is the first report on the formation of *F. betulina* EPs *in vitro*. Screening for EP production enabled the selection of the most promising strain, *F. betulina* 311, and identified SDB as a suitable medium for its growth. This unique ability of *F. betulina* 311 to biosynthesize extracellular biopolymer at a higher level may be genetically determined as an adaptive

property for survival in colder climatic conditions, as this strain was isolated from the fruiting body collected in a boreal habitat.

The studied 22 strains of *F. betulina* were capable of producing EPs with significant variations, depending on the liquid media used: 0.07-0.22 g/L (GPYB), 0.02-2.20 g/L (SDB), 0.12-1.68 g/L (PDB), 0.09-1.12 g/L (MEB). The media supporting EP production of *F. betulina* strains can be ranked as PDB>SDB>MEB>GPYB. Dextrose and starch as carbon sources and the presence of peptone as a nitrogen source in the media used were favorable for the synthesis of EPs by *F. betulina* strains. PDB medium was also suitable for EP production by *Grifola frondosa* [49], *Clavariadelphus truncatus*, *Cerrena unicolor*, *Coprinus comatus*, *Ganoderma carnosum*, *Lenzites betulina*, *Lentinus strigosus*, and *Laetiporus sulphureus* [50].

Our results showed that EP production significantly depended on the strain of *F. betulina* and the cultivation media used. The results obtained agree with the findings of studies that showed that the composition of a medium can significantly affect EP biosynthesis [50-52]. Significant differences in the amount of synthesized polysaccharides have been established in different studies: 0.34-16.68 g/L [53] and 3.4-6.8 g/L [54] depending on fungus species and incubation time; 0.1-1.1 g/L depending on fungal species, incubation period, and shaking or non-shaking conditions [55]; 0.12-10.90 g/L [50] depending on the specific basidiomycetes and their cultivation conditions (including the composition of the medium); 1.95-20.97 mg/L [56] depending on the fungal species.

EP production by *F. betulina* strains is comparable to that of other basidiomycete species such as *Antrodia camphorata*, *A. cinnamomea*, *Phellinus igniarius*, *Pleurotus dryinus*, *P. pulmonarius* [50], *Ganoderma applanatum*, *G. lucidum* [50,57]; *Grifola frondosa*, and *Schizophyllum commune* [55,57], *Polyporus* sp., *P. florida* [55]; *Pleurotus sajor-caju* [50,55]; *Auricularia delicata*, *Pleurotus ostreatus, Pycnoporus sanguineus,* and *Trametes trogii* [57]. EP production by *F. betulina* strains is higher than in the cultivation of *Agaricus subrufescens*, *P. ostreatus*, *P. eryngii*, and *Lentinula edodes* [56], but lower compared to *Agaricus nevoi*, *Auricularia auricula*, *Fomes fomentarius*, *Grifola frondosa*, *Inonotus levis*, *Lentinus edodes*, *Phellinus* sp., *P. gilvus*, and *Trametes versicolor* [50]. It should be noted that the amount of EPs in *F. betulina* can be further increased by optimizing the cultivation conditions, as has been shown in studies with other basidiomycetes like *Ganoderma lucidum* [58], *Hericium coralloides* [59], *Pleurotus citrinopileatus* [60], and *Trametes versicolor* [61].

Fungal growth and development exhibit species and strain-specific characteristics. Therefore, cultivation protocols suitable for a specific culture or isolate should be developed. Each stage of cultivation should aim to obtain the best results from the culture at a minimum cost. From an economic standpoint, a significant advantage is the potential to use a single cultivation medium for both mushroom growth and the biosynthesis of biological substances.

The Pearson correlation coefficient values showed a weak correlation between biomass and the EP content in the two media (SDB and MEB). This result was also suitable for the selected *F. betulina* 311. Mykhaylova et al. [62] also noted a correlation between biomass and EP production under specified conditions. However, the maximum mycelial growth and the highest output of bioactive extracellular metabolites are often achieved under different cultivation conditions [19,20,63-66]. A lack of correlation between biomass and EP production in the cultivation of 51 different basidiomycete species has been reported in GPYB [53]. In addition, various strains of *Schizophyllum commune*, *Pycnoporus sanguineus,* and *Trametes villosa* showed different biomass and EP production results. A similar tendency was also found for other strains of *Agaricus subrufescens*, *Pleurotus ostreatus*, and *Lentinula edodes* [56].

CONCLUSIONS

The absence of a universal protocol or basic cultivation conditions suitable for all macrofungi to ensure the desired biomass and EP productivity underscores the need for screening efforts. Our results point to strain-specific dependence and the importance of medium selection for growth and EP production. All studied strains of *F. betulina* can grow and produce EPs at different levels depending on the medium used.

Strain-specific characteristics of *F. betulina* growth and EP production were observed on different media. The *F. betulina* 311 strain has biotechnological potential due to its robust growth on various agar media, efficient biomass synthesis, and highest production of extracellular biopolymers. Further research is necessary to enhance EP production from this strain, elucidate the composition and structures of the EPs, and identify valuable biological activities associated with EPs.

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Conflict of interest disclosure: There are any potential conflicts of interest.

Data availability: Data underlying the reported findings have been provided as a raw dataset available here: https://www.serbiosoc.org.rs/NewUploads/Uploads/Kizitska%20et%20al_Raw%20Dataset.pdf

REFERENCES

1. Lemieszek ML, Langner E, Kaczor J, Kandefer-Szerszen M, Sanecka B, Mazurkiewicz W, Rzeski W. Anticancer effect of fraction isolated from medicinal Birch polypore mushroom, *Piptoporus betulinus* (Bull.: Fr.) P. Karst (Aphyllophoromycetideae): *in vitro* studies. Int J Med Mushrooms. 2009;11(4):351-64. https://doi.org/10.1615/IntJMedMushr.v11.i4.20
2. Grienke U, Zöll M, Peintner U, Rollinger JM. European medicinal polypores – a modern view on traditional uses. J Ethnopharmacol. 2014;154(3):564-83. https://doi.org/10.1016/j.jep.2014.04.030
3. Guthmann J. Medicinal mushrooms: a portrait of the world's most important species. 2nd ed. Wiebelsheim: Quelle & Meyer Verlag; 2016. German.
4. Pleszczyńska M, Lemieszek MK, Siwulski M, Wiater A, Rzeski W, Szczodrak J. *Fomitopsis betulina* (formerly *Piptoporus betulinus*): the Iceman's polypore fungus with modern biotechnological potential. World J Microbiol Biotechnol. 2017;33(83):1-12. https://doi.org/10.1007/s11274-017-2247-0
5. Gründemann C, Reinhardt JK, Lindequist U. European medicinal mushrooms: do they have potential for modern medicine? An update. Phytomedicine. 2019;66:153131. https://doi.org/10.1016/j.phymed.2019.153131
6. Gafforov Y, Deshmuk SK, Verekar SA, Tomšovský M, Yarasheva M, Chen JJ, Langer E, Rapior S. *Fomitopsis betulina* (Bull.) B.K. Cui, M.L. Han & Y.C. Dai; *Fomitopsis pinicola* (Sw.) P. Karst. – Fomitopsidaceae. In: Khojimatov OK, Gafforov Y, Bussmann RW, editors. Ethnobiology of Uzbekistan. Ethnobiology. Cham: Springer; 2023. p. 1085-101. https://doi.org/10.1007/978-3-031-23031-8_108
7. Rutalek R. Ethnomycology – An Overview. Osterr Z Pilzkd. 2002;11:79-94. German.
8. Papp N, Rudolf K, Bencsik T, Czégényi D. Ethnomycological use of *Fomes fomentarius* (L.) Fr. and *Piptoporus betulinus* (Bull.) P. Karst. in Transylvania, Romania. Genet Resour Crop Evol. 2017;64:101-11. https://doi.org/10.1007/s10722-015-0335-2
9. Zwolińska K. Evaluation of anticancer activity of extracts from birch polypore *Piptoporus betulinus* (Bull. ex Fr.) P. Karst. [dissertation]. Lublin: Maria Curie-Skłodowska University; 2004.
10. Cyranka M, Graz M, Kaczor J, Kandefer-Szerszen M, Walczak K, Kapka-Skrzypczak L, Rzeski W. Investigation of antiproliferative effect of ether and ethanol extracts of birch polypore medicinal mushroom, *Piptoporus betulinus* (Bull.:Fr.) P. Karst. (higher Basidiomycetes) *in vitro* grown mycelium. Int J Med Mushrooms. 2011;13(6):525-33. https://doi.org/10.1615/intjmedmushr.v13.i6.40
11. Sułkowska-Ziaja K, Szewczyk A, Galanty A, Gdula-Argasińska J, Muszyńska B. Chemical composition and biological activity of extracts from fruiting bodies and mycelial cultures of *Fomitopsis betulina*. Mol Biol Rep. 2018;45(6):2535–44. https://doi.org/10.1007/s11033-018-4420-4
12. Khalilov Q, Li L, Liu Y, Tohtahon Z, Chen X, Aisa HA, Yuan T. Piptolinic acids F-J, five new lanostane-type triterpenoids from *Piptoporus betulinus*. Nat Prod Res. 2018;33(21):3044-51. https://doi.org/10.1080/14786419.2018.1516218
13. Weber LA, Puff C, Kalbitz J, Kietzmann M, Feige K, Bosse K, Rohn K, Cavalleri JV. Concentration profiles and safety of topically applied betulinic acid and NVX-207 in eight healthy horses – A randomized, blinded, placebo-controlled, crossover pilot study. J Vet Pharmacol Ther. 2020;44(1):47-57. https://doi.org/10.1111/jvp.12903
14. Schlegel B, Luhmann U, Haertl A, Graefe U. Piptamine, a new antibiotic produced by *Piptoporus betulinus* Lu 9-1. J Antibiot (Tokyo). 2000;53(9):973–4. https://doi.org/10.7164/antibiotics.53.973
15. Keller AC, Maillard MP, Hostettmann K. Antimicrobial steroids from the fungus *Fomitopsis pinicola*. Phytochemistry. 1996;41(4):1041-6. https://doi.org/10.1016/0031-9422(95)00762-8
16. Alresly Z, Lindequist U, Lalk M, Porzel A, Arnold N, Wessjohann LA. Bioactive triterpenes from the fungus *Piptoporus betulinus*. Rec Nat Prod. 2016;10(1):103-8.
17. Alresly Z. Chemical and pharmacological investigations of *Fomitopsis betulina* (formerly: *Piptoporus betulinus*) and *Calvatia gigantea* [dissertation]. Greifswald: Universität of Greifswald; 2019. https://doi.org/10.13140/RG.2.2.27835.54561

18. Krupodorova TA, Barshteyn VYu, Zabeida EF, Pokas EV. Antibacterial activity of macromycetes mycelia and culture liquid. Microbiol Biotechnol Lett. 2016;44(3):246-53. https://doi.org/10.4014/mbl.1603.03003
19. Krupodorova T, Barshteyn V, Kizitska T, Kvasko H, Andriiash H, Tigunova O. Effect of ultraviolet C irradiation on growth and antibacterial activity of *Fomitopsis betulina* (Bull.) B.K. Cui, M.L. Han and Y.C. Dai. GSC Biol Pharm Sci. 2018;04(03):001–6. https://doi.org/10.30574/gscbps.2018.4.3.0073
20. Krupodorova TA, Barshteyn VYu, Kizitska TO, Pokas EV. Effect of cultivation conditions on mycelial growth and antibacterial activity of *Lentinula edodes* and *Fomitopsis betulina*. Czech Mycol. 2019;71(2):167-86. https://doi.org/10.33585/cmy.71204
21. Verekar SA, Gupta MK, Deshmukh SK. *Fomitopsis betulina* a rich source of diverse bioactive metabolites. In: Sridhar KR, Deshmukh SK editors. Advances in macrofungi. Pharmaceuticals and cosmeceuticals. Boca Raton: CRC Press; 2021. p. 22-66. https://doi.org/10.1201/9781003191278
22. Vunduk J, Klaus A, Kozarski M. Petrovic P, Zizak Z, Niksic M, Van Griensven LJ. Did the Iceman know better? Screening of the medicinal properties of the Birch polypore medicinal mushroom, *Piptoporus betulinus* (higher Basidiomycetes). Int J Med Mushrooms. 2015;17(12):1113-25. https://doi.org/10.1615/intjmedmushrooms.v17.i12.10
23. de Jesus LI, Smiderle FR, Ruthes AC, Vilaplana F, Dal'Lin FT, Maria-Ferreira D, Werner MF, Van Griensven L, Iacomini M. Chemical characterization and wound healing property of a β-D-glucan from edible mushroom *Piptoporus betulinus*. Int J Biol Macromol. 2018;117:1361-6. https://doi.org/10.1016/j.ijbiomac.2017.12.107
24. Grunewald F, Steinborn C, Huber R, Wille R, Meier S, Alresly Z, Lindequist U, Gründemann C. Effects of Birch polypore mushroom, *Piptoporus betulinus* (Agaricomycetes), the "Iceman's Fungus", on human immune cells. Int J Med Mushrooms. 2018;20(12):1135-47. https://doi.org/10.1615/IntJMedMushrooms.2018029154
25. Sofrenić I, Anđelković B, Todorović N, Stanojković T, Vujisić L, Novaković M, Milosavljević S, Tešević V. Cytotoxic triterpenoids and triterpene sugar esters from the medicinal mushroom *Fomitopsis betulina*. Phytochemistry. 2021;181:112580. https://doi.org/10.1016/j.phytochem.2020.112580
26. Wiater A, Paduch R, Plesczyńska M, Pŕochniak K, Choma A, Kandefer-Szerszeń M, Szczodrak J. α-(1 → 3)-D-Glucans from fruiting bodies of selected macromycetes fungi and the biological activity of their carboxymethylated products. Biotechnol Lett. 2001;33:787-95. https://doi.org/10.1007/s10529-010-0502-7
27. Mykhaylova OB. Morphological and cultural properties of a medicinal mushroom, *Piptoporus betulinus* (*Basidiomycetes*), on nutrient agar media. Ukr Bot J. 2014;71(5):603-9. Ukrainian. https://doi.org/10.15407/ukrbotj71.05.603
28. Badalyan SM, Gharibyan NG. Characteristics of mycelial structures of different fungal collections – Yerevan: YSU Press, 2017, p. 176.
29. Mykchaylova O, Lomberg M, Krasinko V. Biotechnological basis of intensive cultivation of medicinal mushroom *Fomitopsis betulina* (Fomitopsidaceae, Polyporales). Sci Works NUFT. 2021;27(1):32-41. Ukrainian.
30. Mensah D, Obodai M. Morphological characteristics of mycelial growth of two strains of the indigenous medicinal mushroom, *Lentinus squarrosulus* Mont. (Singer), on solid media. Afr J Agric Res. 2014;9(23):1753-60. https://doi.org/10.5897/AJAR2013.8340
31. Zerva A, Tsafantakis N, Topakas E. Evaluation of Basidiomycetes wild strains grown in agro-industrial residues for their anti-tyrosinase and antioxidant potential and for the production of biocatalysts. Fermentation (Basel). 2021;7(1):19. https://doi.org/10.3390/fermentation7010019
32. Krupodorova T, Barshteyn V, Tsygankova V, Sevindik M, Blume Y. Strain-specific features of *Pleurotus ostreatus* growth in vitro and some of its biological activities. BMC Biotechnol. 2024;24:9. https://doi.org/10.1186/s12896-024-00834-9
33. Bisko N, Lomberg M, Mykhaylova O, Mytropolska N. IBK Mushroom Culture Collection [Internet]. Version 1.8. Copenhagen (Denmark): GBIF Norway; 2024 Feb 24 [cited 2024 Apr 29]. Available from: https://doi.org/10.15468/dzdsqu
34. Weis AL, Solomko EF, Buchalo AS, Wasser SP, Mitropolskaya NYu, Grigansky APh, Gorovits EL. Cultural study and illudin S production of medicinal mushroom *Omphalotus olearius* (DC.: Fr.) Fay. (Agaricales s.l.) from Israel. Int J Med Mushrooms. 1999;1(1):93-103. https://doi.org/10.1615/IntJMedMushrooms.v1.i1.80
35. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Anal Chem. 1956;28(3):350-6. https://doi.org/10.1021/ac60111a017
36. Evans JD. Straightforward statistics for the behavioral sciences. Pacific Grove: Brooks/Cole Pub. Co.; 1996.
37. Badalyan SM, Gharibyan NG, Iotti M, Zambonelli A. Morphological and ecological screening of different collections of medicinal white-rot bracket fungus *Ganoderma adspersum* (Schulzer) Donk (Agaricomycetes, Polyporales). Ital J Mycol. 2019;48:1-15. https://doi.org/10.6092/issn.2531-7342/9092
38. Krupodorova T, Barshteyn V, Al-Maali G, Sevindik M. Requirements for vegetative growth of *Hohenbuehelia myxotricha* and its antimycotic activity. Pol J Natur Sc. 2022;7(1):75-92.
39. Badalyan S, Gharibyan N, Gianchino C, Iotti M, Zambonelli A. Morphological observation and biomass formation in different edible medicinal *Morchella* collections (Pezizomycetes, Ascomycota). Ital J Mycol. 2023;52(1):50-61. https://doi.org/10.6092/issn.2531-7342/16112
40. Guadarrama-Mendoza PC, del Toro GV, Ramírez-Carrillo R, Robles-Martínez F, Yáñez-Fernández J, Garín-Aguilar ME, Hernández CG, Bravo-Villa G. Morphology and mycelial growth rate of *Pleurotus* spp. strains from the Mexican mixtec region. Braz J Microbiol. 2014; 45(3):861-72. https://doi.org/10.1590/s1517-83822014000300016
41. Dzhagan V, Krupodorova T, Atamanchuk A, Lytvynenko Y, Dzhagan V. Growth and morphological characteristics of some pyrophilous discomycetes in culture. Biosyst Divers. 2023;31(3):282-9. https://doi.org/10.15421/012332
42. Lomberh ML. Medicinal mushrooms in surface and submerged culture [PhD thesis]. Kyiv: M.G. Kholodny Institute of Botany, National Academy of Science; 2005. (In Ukrainian).

43. Krupodorova T, Barshteyn V, Sekan A. Review of the basic cultivation conditions influence on the growth of basidiomycetes. Curr Res Environ Appl Mycol J Fungal Biol. 2021;1:494-531. https://doi.org/10.5943/cream/11/1/34
44. Jaros D, Köbsch J, Rohm H. Exopolysaccharides from Basidiomycota: Formation, isolation and techno-functional properties. Eng Life Sci. 2018;18(10):743-52. https://doi.org/10.1002/elsc.201800117
45. Osemwegie OO, Adetunji CO, Ayeni EA, Adejobi OI, Arise RO, Nwonuma CO, Oghenekaro AO. Exopolysaccharides from bacteria and fungi: current status and perspectives in Africa. Heliyon. 2020;6(6):e04205. https://doi.org/10.1016/j.heliyon.2020.e04205
46. Osińska-Jaroszuk M, Jarosz-Wilkołazka A, Jaroszuk-Ściseł J, Szałapata K, Nowak A, Jaszek M, Ozimek E, Majewska M. Extracellular polysaccharides from Ascomycota and Basidiomycota: production conditions, biochemical characteristics, and biological properties. World J Microbiol Biotechnol. 2015;31:1823-44. https://doi.org/10.1007/s11274-015-1937-8
47. Hamidi M, Okoro OV, Milan PB, Khalili MR, Samadian H, Nie L, Shavandi A. Fungal exopolysaccharides: Properties, sources, modifications, and biomedical applications. Carbohydr Polym. 2022;284:119152. https://doi.org/10.1016/j.carbpol.2022.119152
48. Stoica RM, Moscovici M, Lakatos ES, Cioca LI. Exopolysaccharides of fungal origin: Properties and pharmaceutical applications. Processes. 2023;11(2):335. https://doi.org/10.3390/pr11020335
49. Lee BC, Bae JT, Pyo HB, Choe TB, Kim SW, Hwang HJ, Yun JW. Submerged culture conditions for the production of mycelial biomass and exopolysaccharides by the edible Basidiomycete *Grifola frondose*. Enzym Microb Technol. 2004;35(5):369-76. https://doi.org/10.1016/j.enzmictec.2003.12.015
50. Osińska-Jaroszuk M, Jarosz-Wilkołazka A, Jaroszuk-Ściseł J, Szałapata K, Nowak A, Jaszek M, Ozimek E, Majewska M. Extracellular polysaccharides from Ascomycota and Basidiomycota: production conditions, biochemical characteristics, and biological properties. World J Microbiol Biotechnol. 2015;31(12):1823-44. https://doi.org/10.1007/s11274-015-1937-8
51. Tavares AP, Agapito MS, Coelho MA, Lopes da Silva JA, Barros-Timmons A, Coutinho JA, Xavier AM. Selection and optimization of culture medium for exopolysaccharide production by *Coriolus (Trametes) versicolor.* World J Microbiol Biotechnol. 2005;21:1499-507. https://doi.org/10.1007/s11274-005-7370-7
52. Lin ES, Sung SC. Cultivating conditions influence exopolysaccharide production by the edible Basidiomycete *Antrodia cinnamomea* in submerged culture. Int J Food Microbiol. 2006;108(2):182-7. https://doi.org/10.1016/j.ijfoodmicro.2005.11.010
53. Maziero R, Cavazzoni V, Bononi V L R Screening of basidiomycetes for the production of exopolysaccharide and biomass in submerged culture. Revista de Microbiologia. 1999;30:77-84.
54. Sasidhara R, Bakki V, Thirunalas T Screening of some basidiomycetes for useful polysaccharides. Mushroom Reseearch. 2011;20(2):83-6.
55. Rajput Y, Shit S, Shukla A, Gupta S, Shukla K Screening for exopolysaccharide production from basidiomycetes of Chhattisgarh. CCCRENT Botany 2011, 2(10):11-14
56. Umeo SH, Souza GP, Rapachi PM, Garcia DM, Paccola-Meirelles LD, Valle JS, Colauto NB, Linde GA. Screening of basidiomycetes in submerged cultivation based on antioxidant activity. Genet Mol Res. 2015;14(3):9907-14. https://doi.org/10.4238/2015
57. Montoya S, Sánchez ÓJ, Levin L. Polysaccharide Production by Submerged and Solid-State Cultures from Several Medicinal Higher Basidiomycetes. Int J Med Mushrooms. 2013,15(1): 71–9.
58. Asadi F, Barshan-Tashnizi M, Hatamian-Zarmi A, Davoodi-Dehaghani F, Ebrahimi-Hosseinzadeh B. Enhancement of exopolysaccharide production from *Ganoderma lucidum* using a novel submerged volatile co-culture system. Fungal Biol. 2021;125(1):25-31 https://doi.org/10.1016/j.funbio.2020.09.010
59. Tabibzadeh, F., Alvandi, H., Hatamian-Zarmi, A. et al. Antioxidant activity and cytotoxicity of exopolysaccharide from mushroom Hericium coralloides in submerged fermentation. Biomass Conv. Bioref. 2022. https://doi.org/10.1007/s13399-022-03386-0
60. Wu C-Y, Liang Z-C, Lu C-P, Wu S-H. Effect of carbon and nitrogen sources on the production and carbohydrate composition of exopolysaccharide by submerged culture of pleurotus citrinopileatus, Journal of Food and Drug Analysis. 2008;16(2):6. https://doi.org/10.38212/2224-6614.2364
61. Bolla K, Gopinath BV, Shaheen Syed Zeenat, Singara Charya MA. Optimization of carbon and nitrogen sources of submerged culture process for the production of mycelial biomass and exopolysaccharides by *Trametes versicolor*. Int J Biotechnol Mol.Biol Res. 2010;1(2):15-21.
62. Mykchaylova O, Dubova H, Lomberg M, Negriyko A, Poyedinok N. Influence of low-intensity light on the biosynthetic activity of the edible medicinal mushroom *Hericium erinaceus* (Bull.: Fr.) Pers. *in vitro*. Arch Biol Sci. 2023;75(4):489-501. https://doi.org/10.2298/ABS230821040M
63. Kim YR. Production of polysaccharide by the edible mushroom, *Grifola frondosa*. Mycobiology. 2003;31(4):205-8.
64. Elisashvili V. Submerged cultivation of medicinal mushrooms: bioprocesses and products (Review). Int J Med Mushrooms. 2012;14(3):211-39. https://doi.org/10.1615/intjmedmushr.v14.i3.10
65. Bisko N, Mustafin K, Al-Maali G, Suleimenova Z, Lomberg M, Narmuratova Z, Mykchaylova O, Mytropolska N, Zhakipbekova A. Effects of cultivation parameters on intracellular polysaccharide production in submerged culture of the edible medicinal mushroom *Lentinula edodes*. Czech Mycol. 2020;72(1):1-17. https://doi.org/10.33585/cmy.72101
66. Narmuratova Zh, Bisko N, Mustafin K, Al-Maali G, Kerner A, Bondaruk S, Suleimenova Zh, Kalieva A, Akhmetsadykov N, Zhakipbekova A, Lomberg M. Biological activity of edible medicinal mushrooms of the genus *Hericium*. Turk J Biochem. 2023;48(3):290-7. https://doi.org/10.1515/tjb-2022-0235