Fungal Biology and Biotechnology

RESEARCH

Open Access

Enhancement of antioxidant activity and total phenolic content of *Fomitopsis pinicola* mycelium extract

Tetiana Krupodorova[1*], Victor Barshteyn[1], Veronika Dzhagan[2], Andrii Pluzhnyk[2,3], Tetiana Zaichenko[1] and Yaroslav Blume[4]

Abstract

Background *Fomitopsis pinicola* is one of the most common fungi found in temperate zone of Europe, widely distributed spread in Asia and North America. Fungus has a wide range of therapeutic activity: antitumor, antimicrobial, anti-inflammatory, antidiabetic, antifungal, hepatoprotective, hemostatic action. A number of studies have confirmed the significant antioxidant activity of *F. pinicola* fruiting bodies. However, the controlled cultivation conditions that influence fungal growth and metabolite production of *F. pinicola*, particularly the mycelial growth and biosynthesis of metabolites in its culture broth, as well as the antioxidant activity of its mycelium, remain poorly understood.

Results This study investigated the impact of cultivation conditions on *F. pinicola* mycelium growth, phenols synthesis and antioxidant activity. Difference in the biosynthetic activity of *F. pinicola* under tested cultivation conditions was established. A highest value of 2,2-diphenyl-1-picryl-hydrazyl (DPPH•) inhibition (78.2 ± 0.9%) was found for a mycelium cultivated at 30 °C, while cultivation at a lower temperature (20 °C) was suitable for biomass growth (8.5 ± 0.3 g/L) and total phenolic content (TPC) 11.0 ± 0.6 mg GAE/g. Carbon and nitrogen sources in a cultivation broth significantly influenced the studied characteristics. Xylose supported the highest DPPH• inhibition (89.91 ± 0.5%) and TPC (16.55 ± 0.4 mg GAE/g), while galactose yielded the best biomass (4.0 ± 0.3 g/L). Peptone was the most effective nitrogen source for obtaining the mycelium with high potential of DPPH• radical inactivation (90.42 ± 0.5%) and TPC (17.41 ± 0.5 mg GAE/g), while the maximum biomass yield (7.8 ± 0.6 g/L) was found with yeast extract in cultivation medium. *F. pinicola* demonstrated the ability to grow and produce bioactive metabolites across a wide pH range from 2.5 to 7.5. Shaking cultivation resulted in the highest TPC (21.44 ± 0.10 mg GAE/g), though the same level of antioxidant activity (93%) was achieved under both shaking and static cultivation on the 7th and 28th days, respectively.

Conclusion Controlling cultivation parameters makes it possible to regulate the metabolic and biochemical processes of *F. pinicola*, facilitating the balance needed to obtain optimal biomass, phenols and antioxidant activity. The findings show the potential to increase phenol production by 2.25 and 2.23 times under shaking and static conditions, respectively, while maintaining a high level of activity.

*Correspondence:
Tetiana Krupodorova
krupodorova@gmail.com

Full list of author information is available at the end of the article

Keywords Red-belted conk, Mycelium, Cultivation conditions, Extract, DPPH, TPC

Introduction

Macrofungi, particularly basidiomycetes and ascomycetes, have become a subject of great interest to modern biotechnologists. The valuable biological activity of macrofungi, along with their important secondary metabolites, has attracted widespread attention from researchers. The health-improving effects of macrofungi and their potential in preventing various diseases due to their wide range of pharmacological activities - such as anticancer, antioxidant, antimicrobial, antiviral, anti-inflammatory, antihyperlipidemic, antidiabetic, antidepressant, antiallergic, hepatoprotective, neuroprotective, cytotoxic, and immunomodulatory - have been thoroughly covered in numerous recent reviews [1–6]. The wide variety of fungi with medicinal properties is certainly impressive. However, some fungi, like *Ganoderma*, *Cordyceps*, *Pleurotus*, *Trametes*, *Lentinula*, *Hericium*, and *Inonotus* species, are more researched, while others are less studied. *Fomitopsis pinicola* (Sw.) P. Karst. (Red-belted Conk, Red-belted Bracket) attracts attention due to various biological compounds (enzymes, glycosides, heteroglycan, triterpenes, polysaccharides, polyphenols, steroids) and their derivatives, which have beneficial effects, as reviewed by Bishop [7], Zahid et al. [8], and Gafforov et al. [9].

F. pinicola is one of the most common fungi found in temperate zone of Europe, widely distributed spread in Asia and North America. In terms of its ecology, this fungus is a parasite and saprotroph that causes brown rot of mainly coniferous species, as well as wood belonging to the genera of *Alnus* and *Betula* [9].

The fruiting bodies of *F. pinicola* have long been used in folk medicine. To this day, there are references to its useful properties, such as anti-inflammatory and blood-stopping effects, as well as its use for treating liver disorders, headaches, and nausea [10].

Various extracts of the fruiting bodies of *F. pinicola* are being studied by scientists to assess their therapeutic potential. Studies have focused on a very relevant property – its anticancer activity. This activity of *F. pinicola* fruiting bodies has been demonstrated in vitro on various cell lines [11–19] as well as in vivo (14, 15). Some studies investigated the cytotoxic potential of *F. pinicola* on HeLa cells [20, 21] as well as SMMC-7721 cells [21].

F. pinicola has been reported to have a broad spectrum of antimicrobial activity. Extracts of its fruiting bodies inhibited the growth of various bacteria: *Escherichia coli* [17, 22], *Staphylococcus aureus* [17, 22, 23], *Bacillus subtilis* [22–24], *B. cereus* [25], *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* [22]. *F. pinicola* mycelium has suppressed the growth of *B. subtilis* and *E. coli*, while its culture liquid has inhibited the growth of these two bacteria as well as *S. aureus* [26]. Extracts of its fruiting bodies have also exhibited activity against fungal pathogens: *Fusarium inflexum* and *F. heterosporium* [27], *Saccharomyces cerevisiae*, *Candida albicans*, *Penicillium chrysogenum*, and *Aspergillus fumigatus* [22]. The antifungal activity of *F. pinicola* mycelium has been observed in dual culture, showing complete overgrowth on the mycelium of *Chrysosporium keratinophilum* and *Penicillium griseofulvum* [28], as well as on strains of *Candida albicans* and *Pichia kudriavzevii* [29]. Additionally, it has exhibited partial replacement on *Aspergillus niger* and a deadlock after mycelial contact with *Penicillium polonicum* [30].

Some reports have been devoted to the anti-inflammatory [31], chemo-preventive [16], and antidiabetic [32] properties of *F. pinicola* carpophore extracts. It has also been found that *F. pinicola* extract is a powerful tool for the prevention and therapy of injured liver and kidney cells [33].

Another important topic of research has been the study of the antioxidant potential of fungi. The source of energy for biological processes in living organisms is the oxidation process. Reactive nitrogen species (RNS) and, first of all, reactive oxygen species (ROS) are free radicals that can destroy healthy cells in the body. Excess production of ROS or loss of natural antioxidant protection, or in other words, an imbalance in the formation of free radicals, is known as oxidative stress. This excess of ROS can cause oxidation of lipids, DNA or proteins, being a major factor in aging and the development of a number of different degenerative diseases in humans [34]. Prevention and treatment of these diseases, including cardiovascular, oncological and central nervous system diseases, can be achieved using biologically active substances with antioxidant potential contained in fungi. The significant antioxidant activity of *F. pinicola* fruiting bodies has been established by various methods in numerous studies [12, 16, 18, 19, 35–38]. Among the various methods for determining the antioxidant potential of biological samples, the rapid, simple and inexpensive DPPH• method has been noted [39, 40]. Phenols and polyphenols scavenge reactive oxygen species (ROS) in a variety of ways, including inactivating metals, scavenging oxygen, or inhibiting free radicals [41]. The widely used Folin-Ciocalteu method has offered high sensitivity and has measured a broad spectrum of phenolic compounds, making it well-suited for analyzing complex phenolic mixtures in various biological samples using common lab equipment [42–44]. Various phenolic compounds such as epicatechin, catechin and quercetin dihydrates [36], gallic acid [19, 36], p-hydroxybenzoic acid, protocatechuic

acid, vanillin [19], chlorogenic acid, syringic acid, ellagic acid [45, 46] have been found in the extracts of *F. pinicola* fruiting bodies. Polysaccharides isolated from *F. pinicola* mycelium and its fermentation broth have also been shown to antioxidant activity [12].

Controlled culture conditions have been found to affect fungal growth and metabolite production. However, the mycelium growth of *F. pinicola*, as well as metabolite biosynthesis in its culture broth, are poorly understood. The influence of culture conditions on *F. pinicola* growth [46–48], exopolysaccharide [46], and endoglucanase [49] production were investigated by submerged cultivation. This research has aimed at assessing the impact of cultivation conditions on *F. pinicola* growth and the potential to enhance antioxidant activity as well as TPC.

Materials and methods

Strain

Fomitopsis pinicola, srtain 1523, was kindly obtained from the IBK Mushroom Culture Collection of the M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine [50] as pure culture on Malt Extract Agar (MEA, Thermo Fisher Scientific, USA) slant and stored at 4 °C.

The total DNA of *F. pinicola* was extracted following the method described by Dellaporta [51]. The 18 S region of the internal transcribed spacer (ITS) of ribosomal DNA (rDNA) was amplified by polymerase chain reaction (PCR) using specific primers ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) [52].

The PCR products purification was carried out on a commercial basis by Macrogen Europe (Amsterdam, Netherlands). Sequence identity was verified using the Basic Local Alignment Search Tool (BLAST) at the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA). The sequence obtained was submitted to GenBank with the accession number PQ184654.

Inoculum preparation

Mycelium of *F. pinicola* was transferred to Petri dish with Glucose Peptone Yeast Agar (GPYA) contained 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 $MgSO_4 \times 7H_2O$, and 10.0 agar. This agar-free medium (GPYB) was used for submerged cultivation under static and shaking conditions.

Erlenmeyer flasks (250 mL) containing 50 mL of GPYB medium each were sterilized by autoclaving for 15 min at 121 °C. Each flask with medium was inoculated with three 8-mm-diameter mycelial plugs cut with a sterile drill from GPYA Petri dishes from a seven-day-old culture of *F. pinicola* for future surface static cultivation.

Seven-day-old *F. pinicola* mycelium from a GPYA Petri dish was ground using a homogenized MPW-120 (Mechanika Precyzyjna, Warszawa, Poland) under sterile conditions to prepare an inoculum, which was transferred as 5 mL aliquots to each flask for further cultivation on an orbital shaker at 120 rpm. Inoculated flasks were incubated in the dark at 25 °C.

Impact of cultivation conditions by using a single-factor test

To determine the effect of incubation temperature, flasks with GPYB were inoculated with three mycelial discs and incubated for 14 days of cultivation under static conditions in different thermostats at temperatures of 20 °C, 25 °C, and 30 °C.

To determine the effect of incubation time, flasks containing GPYB were inoculated with the prepared inoculum according to the cultivation method used and incubated at 25 °C. Incubation time was evaluated by monitoring the dynamics of fungal growth: was evaluated by monitoring the dynamics of fungal growth: from 7 to 35 days with intervals of 14 days under static conditions, and from 3 to 11 days with intervals of 2 days under constant shaking. The pellet size was measured using a digital CCD camera and analyzed with Optimas image analysis software.

To study the effect of pH levels, flasks containing GPYB were inoculated with three mycelial discs and incubated under static conditions at 25 °C for 14 days at different pH levels (ranging from 2.5 to 7.5, with 0.5 intervals). The GPYB medium was previously adjusted with 1 M HCl and 1 M NaOH and measured using a digital pH meter.

To study the effect of carbon and nitrogen sources, a base medium consisting of (g/L): 10.0 glucose, 0.4 asparagine, 1.0 KH_2PO_4, and 0.5 $MgSO_4 \cdot 7\ H_2O$ was used. Glucose in the base medium was replaced by an equivalent carbon content of the respective carbon source (mannitol, arabinose, xylose, fructose, galactose, dextrose, lactose, maltose, sucrose, cellulose, or soluble starch). Asparagine was similarly replaced with an equivalent content of the respective nitrogen source (sodium nitrate, ammonium nitrate, ammonium sulfate, urea, L-asparagine, or peptone). The pure carbon or nitrogen per liter of medium was calculated based on the molecular weight of the substances and the percentage of the carbon or nitrogen element in the molecule. Flasks with prepared medium were inoculated with three mycelial discs and incubated 14 days of cultivation under static conditions at 25 °C.

Biomass determination

After the incubation period, the mycelium was separated from the medium by filtration through Whatman filter paper No. 4, washed with distilled water, and dried to a constant weight at 85 °C. The growth of fungi during

cultivation was determined as mycelial biomass (g/L) in absolute dry weight (a.d.w.).

Extract preparation

The mycelium separated from the medium was washed with dH_2O, and dried at 60 °C. One g of the crushed mycelium was extracted with 10 mL of ethanol (96%) under orbital shaking (100 rpm) for 48 h at room temperature. The supernatant, obtained after centrifugation for 10 min at 4500 rpm, was collected and filtered through a 25 µm pore size filter (class 4 filter paper). The obtained sample was kept at 4 °C not more than one week prior to evaluation for antioxidant activity (AOA) and total phenolic content (TPC).

Antioxidant activity and total phenolic content determination

Antioxidant activity was determined by application of commonly used a stable free radical such as 2,2-diphenyl-1-picryl-hydrazyl (DPPH•) [39] and expressed the inhibition percentage of DPPH• scavenging. Ascorbic acid was used as a positive control, dH_2O and the extract without DPPH• as a negative control. The TPC was measured using the Folin–Ciocalteu method [42]. The TPC was determined using the gallic acid as the standard for quantitatively estimating phenolic compounds in the sample and is expressed as gallic acid equivalents (GAE) in milligrams per gram of the sample. Gallic acid was used as a positive control, and all reagents without the extract as a negative control. The detailed procedure of both analyses was presented in our previous study [53].

Statistical analysis

The average results of all studies were obtained in three replications, each of which included at least three studies. The values were expressed as mean ± standard deviation (SD), and differences at p-value ≤ 0.05 were assumed significant calculated using analysis of variance (ANOVA) with the Excel statistical package. The Pearson correlation coefficients (r) for biomass, TPC, and AOA under different cultivation conditions of *F. pinicola* were determined using the online statistical software available at https://www.statskingdom.com/.

Results

The effect of incubation temperature on fungal biomass, TPC and AOA

Experiments with pure cultures of *F. pinicola* were performed under different basic cultivation conditions. A difference in the *F. pinicola* biomass, TPC and AOA was observed under the tested incubation temperatures, as shown in Fig. 1 (A, B). The highest DPPH• inhibition (78.2 ± 0.9%) was found for extract of mycelium cultivated at 30 °C, while an incubation temperature of 20 °C was more suitable for biomass production (8.5 ± 0.3 g/L) and TPC (11.0 ± 0.6 mg GAE/g). After 14 days of cultivation, the pH of the medium decreased from 6 to 3, remaining consistent regardless of the cultivation temperature (Fig. 1A).

The effect of incubation period on fungal biomass, TPC and AOA

A noticeable difference in biomass, TPC and AOA of *F. pinicola* under shaking and static conditions in the growth dynamics was observed (Fig. 2). Under shaking conditions, the highest amount of mycelial biomass was recorded on the 9th day of cultivation (Fig. 2A). Seven days of incubation was optimal for achieving the best DPPH• activity (Fig. 2B). The maximum TPC was observed on the 5th and 7th days, with no significant statistical difference under shaking conditions (Fig. 2A). Under static conditions, the highest amount of mycelial biomass was recorded on the 9th day of cultivation while the best TPC (Fig. 2C) and AOA were observed on the 28th day of cultivation (Fig. 2D). It should be noted that shaking resulted in the highest TPC (21.44 ± 0.10 mg

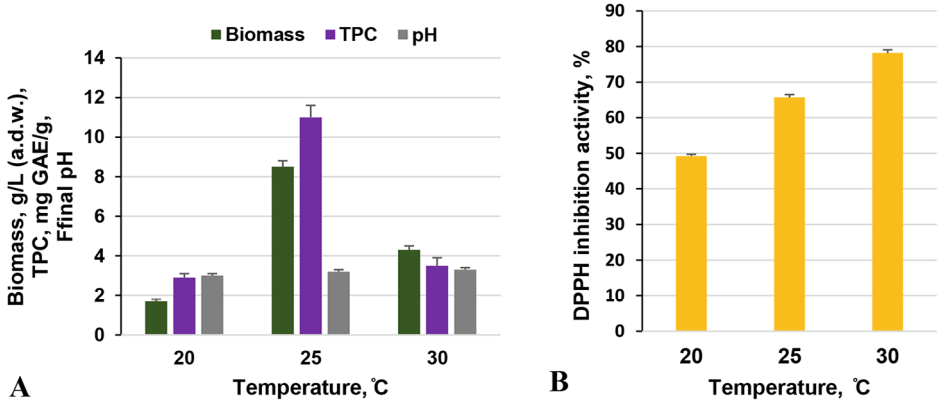

Fig. 1 Effect of temperature on *F. pinicola* biomass production, TPC, final pH (**A**) and DPPH radical scavenging activity (**B**) on 14th day of static cultivation. Bars represent the standard deviation (SD) ($n = 3$, biological replicates)

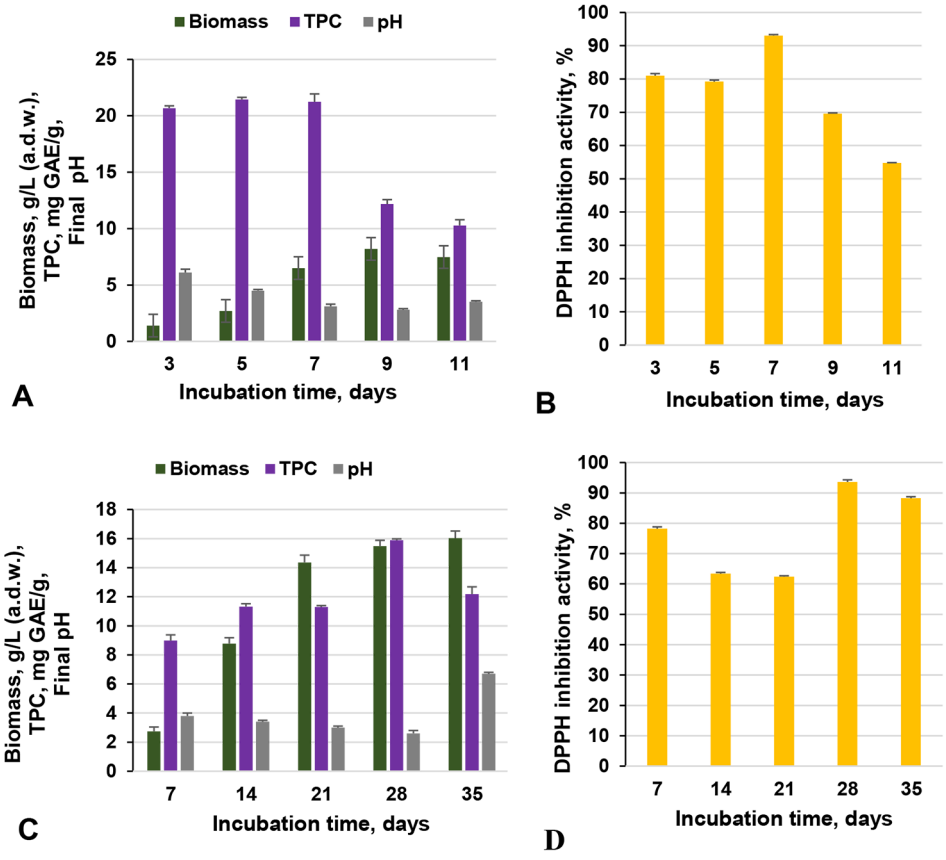

Fig. 2 Effect of incubation period on *F. pinicola* biomass production, TPC, final pH (**A**, **C**) and DPPH radical scavenging activity (**B**, **D**) under shaking and static conditions, respectively. Bars represent the standard deviation (SD) ($n = 3$, biological replicates)

GAE/g) (Fig. 2A), but the same level of AOA (93%) was observed under both shaking and static cultivation on the 7th and 28th days, respectively (Fig. 2B and D). This result is significant because it suggests that while shaking enhances phenolic extraction, the antioxidant capacity is not influenced by the cultivation method, indicating that factors other than phenolic content might contribute to AOA.

During fungal growth, the pH of the culture medium changed (Fig. 2A and C). The range of pH changed in the culture media under both shaking and static conditions was similar, but the lowest pH was observed on day 9 (pH = 2.8) when grown on a shaker, and on day 28 (pH = 2.6) under static conditions.

The effect of cultivation condition on the morphology of fungus

The morphology of *F. pinicola* differed significantly during submerged cultivation, depending on the presence or absence of agitation. Under shaking conditions, *F. pinicola* grew in the form of compact spherical granules (pellets) consisting of clusters of hyphae of varying sizes (from 0.1 to 0.5 mm in diameter), depending on the duration of cultivation (Fig. 3A-C). Under static conditions, *F. pinicola* formed a uniform mycelial mat with a consistent texture and a white-cream color, with pubescent mycelium created by aerial hyphae on the surface of the liquid media (Fig. 3D).

The effect of carbon courses on fungal biomass, TPC and AOA

Various commonly used carbon sources were selected for comparative studies to determine which ones promote rapid growth and which contribute to metabolite synthesis. A significant influence of carbon sources on the studied characteristics of obtained mycelium was observed (Fig. 4). Xylose supported the highest DPPH• inhibition (89.91 ± 0.5%) (Fig. 4B) and phenols production (16.55 ± 0.4 mg GAE/g), while the best biomass yield (4.0 ± 0.3 g/L) was achieved with galactose (Fig. 4A). A similar biosynthetic potential (in terms of biomass production and TPC) was noted when using fructose, dextrose, and galactose. The final pH remained relatively stable, varying slightly from 2.8 to 3.1 (Fig. 4A).

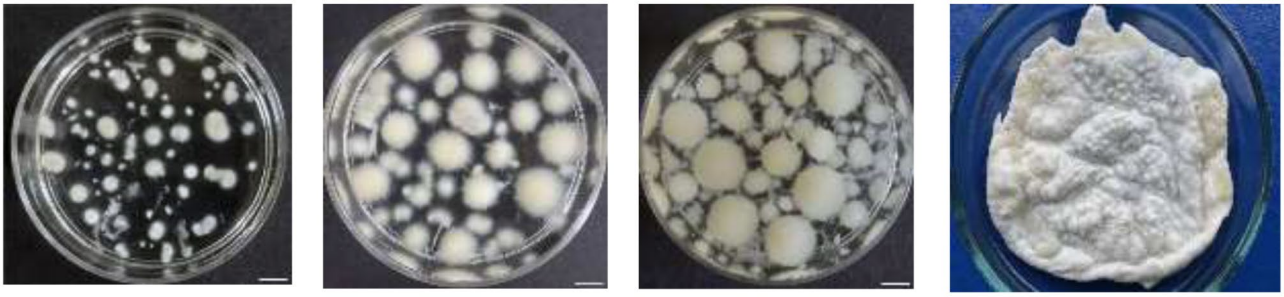

Fig. 3 Morphology of *F. pinicola* (for all three biological replicates) in the form of pellets under shaking on 3 (**A**), 7 (**B**), 11 (**C**) days and of mycelial mat under static conditions on 28 days of the growth (**D**) on glucose peptone yeast broth, scale bar = 1 cm (**A**, **B**, **C**), 2 cm (**D**)

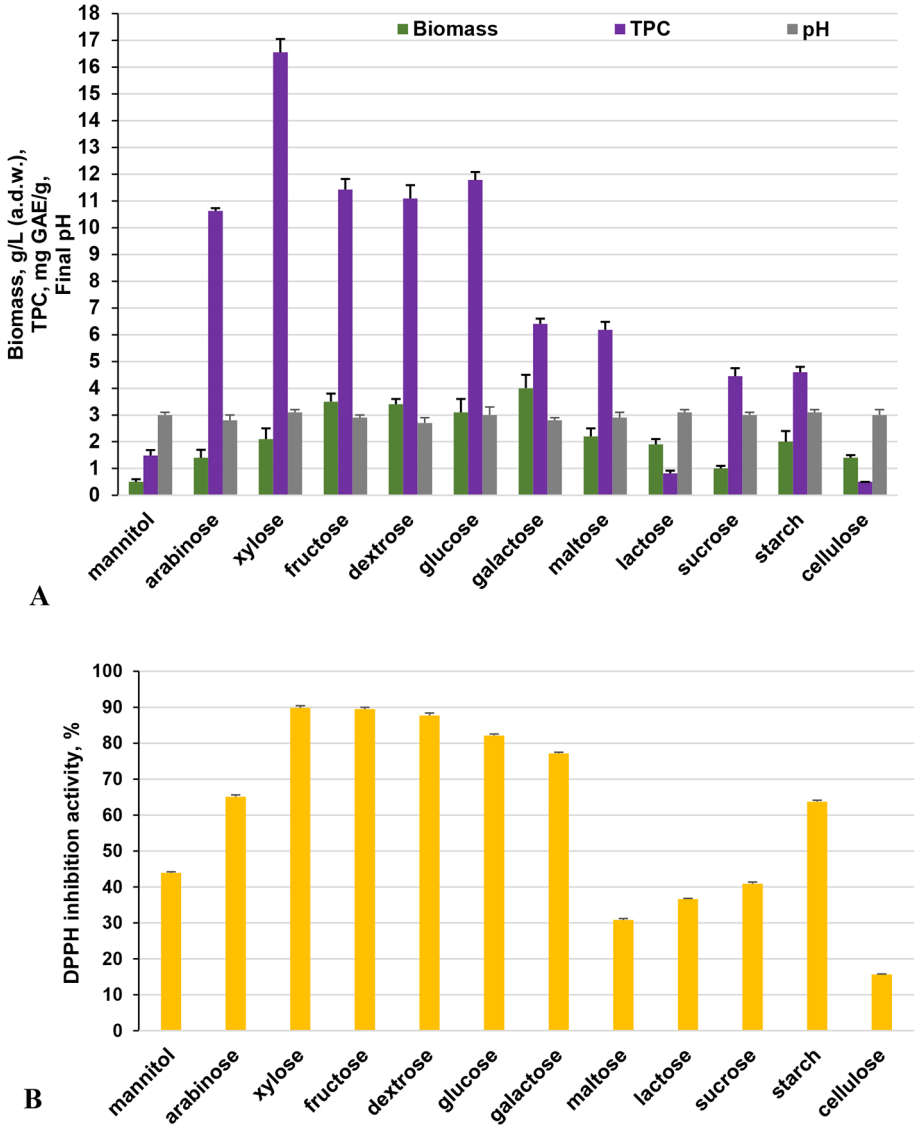

Fig. 4 Effect of carbon sources (at the same concentration 34.83% of carbon in base medium) on *F. pinicola* biomass production, TPC, final pH (**A**) and DPPH radical scavenging activity (**B**) under static conditions. Bars represent the standard deviation (SD) ($n = 3$, biological replicates)

The effect of nitrogen sources on fungal biomass, TPC and AOA

A significant effect of nitrogen sources on the studied characteristics was also found. Peptone was the most effective for DPPH• radical inactivation (90.42±0.5%) (Fig. 5B) and TPC (17.41±0.5 mg GAE/g), while the maximum biomass yield (4.0±0.3 g/L) was achieved with yeast extract (Fig. 5A). The final pH varied slightly, ranging from 2.7 to 2.9 (Fig. 5A).

The effect of initial pH on fungal biomass, TPC and AOA

F. pinicola was able to grow and produce biomass with AOA and TPC across a wide pH range from 2.5 to 7.5 (Fig. 6). It is noteworthy that the studied fungus also demonstrated the aforementioned ability in strong acidic conditions. A very low pH of 2.5 was optimal for AOA (91.61±0.2%), TPC (31.92±0.5 mg GAE/g), as well as biomass production (10.5±0.5 g/L). The final pH showed only slight variation, ranging from 2.6 to 3.6.

Correlation between fungal biomass, TPC, and AOA

The Pearson coefficient (r) was used to assess the potential correlation between biomass, TPC, and AOA in *F. pinicola*. The results demonstrated a positive relationship in all trials (Table 1). A very strong correlation (r=0.80−1.0) was found between TPC and AOA under most of the investigated conditions, including variations in carbon and nitrogen sources, static and shaking

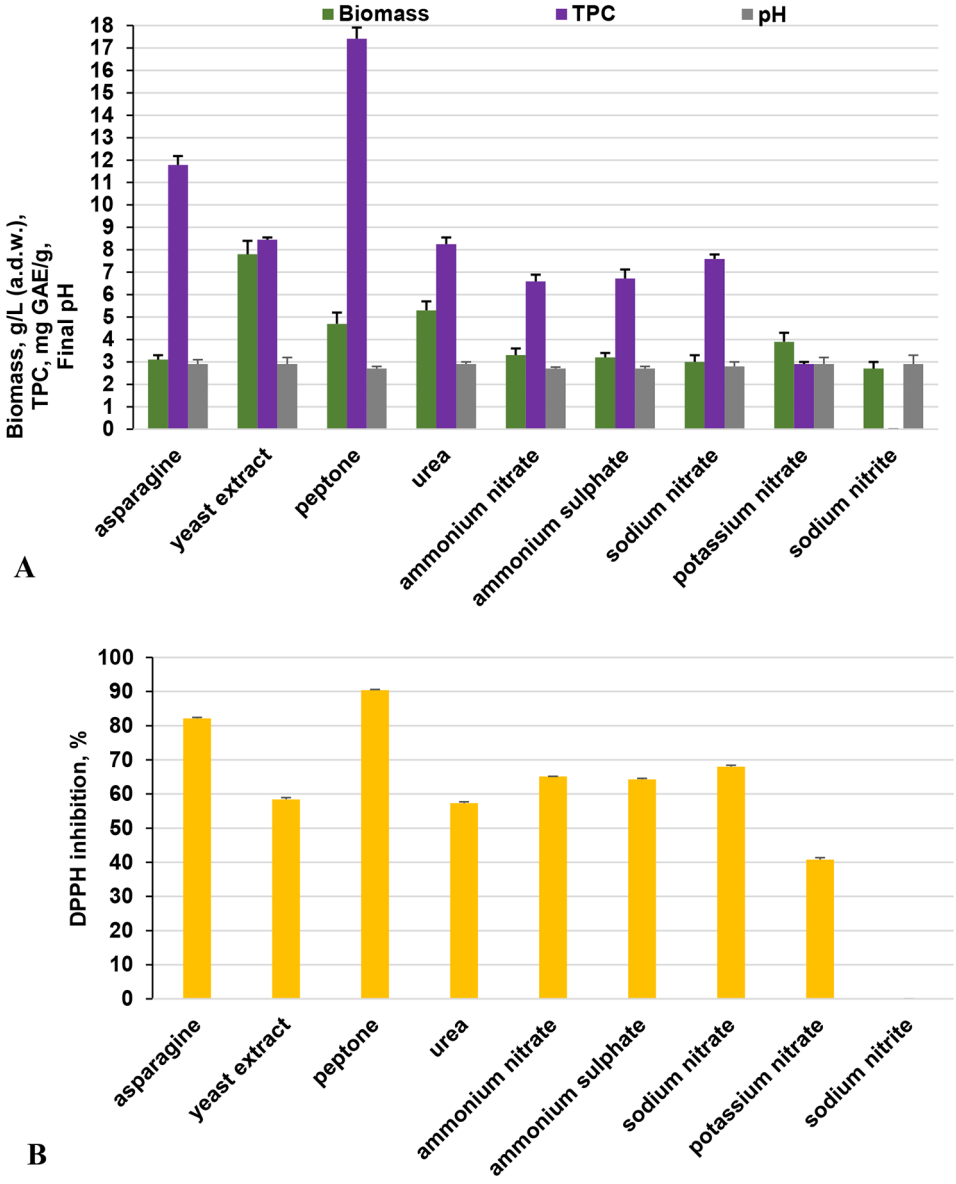

Fig. 5 Effect of nitrogen sources (at the same concentration 0.71% of nitrogen in base medium) on *F. pinicola* biomass production, TPC, final pH (**A**) and DPPH radical scavenging activity (**B**) under static conditions. Bars represent the standard deviation (SD) (n=3, biological replicates)

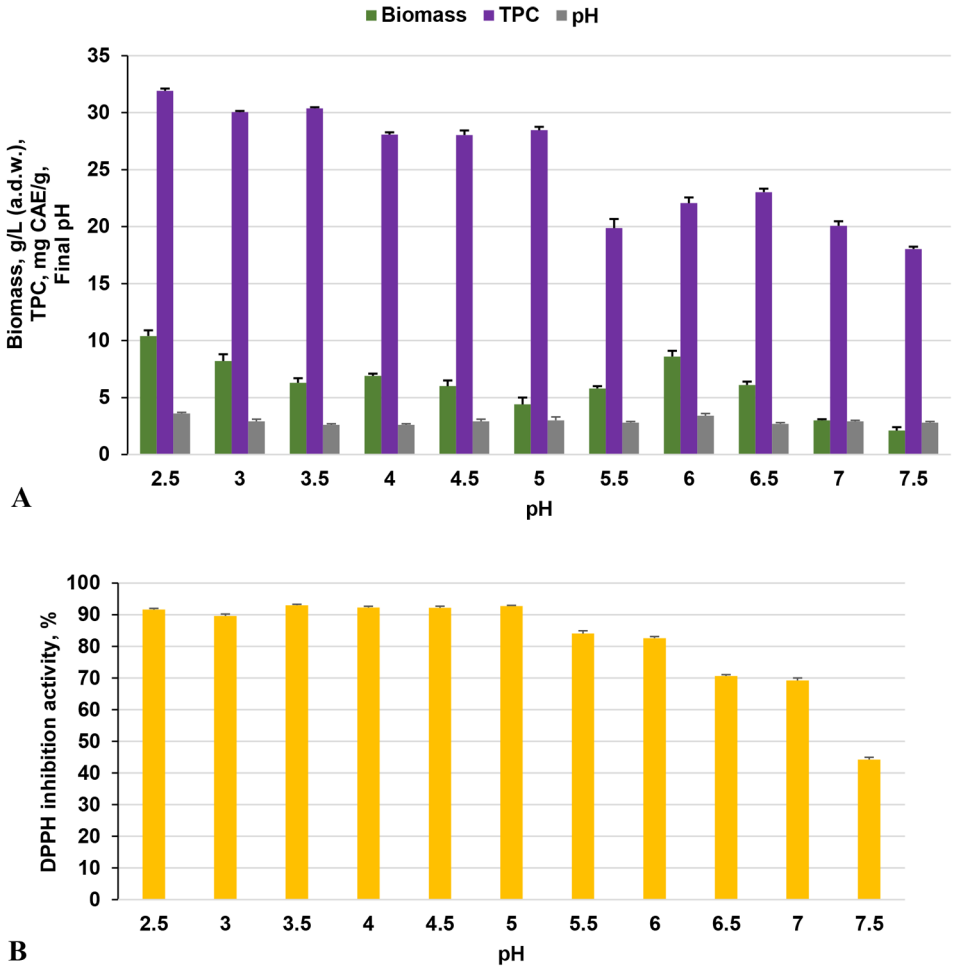

Fig. 6 Effect of initial pH on *F. pinicola* biomass production, TPC (**A**) and DPPH radical scavenging activity (**B**) under static conditions. Bars represent the standard deviation (SD) ($n = 3$, biological replicates)

Table 1 Coefficient (r) established between biomass, TPC, and AOA of *F. pinicola*

Correlation	Temperature	Sources		pH	Cultivation	
		carbon	nitrogen		static	shaking
Biomass/TPC	0.9486	0.4916	0.2919	0.6281	0.8445	0.1703
Biomass/AOA	0.4513	0.6731	0.1713	0.6522	0.7143	0.4447
TPC/AOA	0.1456	0.8451	0.8937	0.8002	0.9334	0.9531

conditions, and pH. Biomass also showed a strong correlation with TPC under specific conditions, such as temperature and static cultivation.

Key cultivation condition of fungal for TPC and AOA

Single-factor influence analysis allowed us to establish the key parameters necessary for the cultivation of *F. pinicola* to optimize growth, TPC, and AOA. By adjusting critical factors, we were able to enhance the TPC and AOA of the studied fungus. Specifically, replacing glucose in the nutrient medium with xylose, lowering the initial pH to 3.5, and cultivating the fungus at 30 °C, both with and without agitation, significantly increased TPC yield while maintaining high AOA (Table 2).

Table 2 Effect of key cultivation conditions of *F. pinicola* on AOA and TPC

Cultivation conditions (30 °C, xylose, pH 3.5)	TPC, mg GAE/g	DPPH• inhibition, %
7 days under shaking condition	48.3 ± 0.67	93.13 ± 0.40
28 days under static condition	37.0 ± 0.59	92.00 ± 0.21

Our experiments demonstrated that different cultivation conditions significantly influenced the biomass production, TPC, and AOA of *F. pinicola*. Notably, while

shaking enhanced phenolic extraction, however optimal antioxidant activity was achieved across a range of conditions.

Discussion

Recently, increased education and information about the benefits of antioxidants, driven by media, modern studies, and health professionals, have raised consumer awareness and demand. There is a growing shift toward preventive healthcare, with people increasingly focused on maintaining health through diet and dietary supplements. This trend has led to a higher demand for antioxidant-rich foods and supplements. Consumers are becoming more aware of the role of antioxidants in preventing oxidative stress-related diseases, such as cardiovascular diseases, cancer, and neurodegenerative disorders [54, 55]. Also, antioxidants are known for their anti-aging properties [56, 57], which are highly sought after in both the dietary supplement and cosmetics industries. These factors collectively contribute to the increasing demand for natural antioxidants across various industries, including food and beverages, cosmetics, and pharmaceuticals. The use of synthetic antioxidants is becoming less desirable due to potential health risks [58], prompting manufacturers to seek natural alternatives. Additionally, concerns about the environmental and health impacts of synthetic chemicals [59] are further driving the demand for natural antioxidants. The shift towards sustainable and eco-friendly practices has led to a growing preference for natural antioxidants, which are often derived from renewable sources and produced using environmentally friendly methods. Moreover, cultural influences and traditional knowledge from regions with a history of using natural antioxidants (e.g., the Mediterranean diet) are spreading globally, contributing to their increased demand [60]. The clear advantages of natural antioxidants underscore the urgency of identifying effective natural sources.

Macrofungi, including basidiomycetes, produce various biologically active compounds such as polyphenols, polysaccharides, vitamins, ergothioneine, and glutathione, all of which exhibit significant antioxidant properties [61, 62]. These natural compounds protect cells from oxidative stress, making them valuable in both nutritional supplements and pharmaceuticals [63]. Mycelium with antioxidant activity can be incorporated into functional foods, beverages, and dietary supplements. The growing consumer interest in natural and healthy ingredients is creating favorable conditions for the development and increased demand for these products in various markets. Ensuring that mycelium-based products meet safety and efficacy standards is critical to their market success. The use of generally accepted methods of cultivation, such as submerged fermentation and the use of bioreactors, allows obtaining mushroom mycelium with the desired stable properties [64]. Submerged static cultivation (also called as surface cultivation) of basidiomycete mycelium offers several advantages and involves specific biotechnological aspects that make it a valuable method for producing fungal biomass and bioactive compounds. This cultivation can better mimic the natural environment of basidiomycetes, potentially leading to the production of compounds that are not synthesized or are produced in lower amounts in liquid cultures. It was shown that static cultivation of *Xylaria feejeensis* was more suitable for manifestation of antioxidant activity as well as for TPC formation [65]. Also, submerged static cultivation typically requires less sophisticated equipment than submerged fermentation, making it a cost-effective option for producing mycelium, especially at a smaller scale. Despite the available biotechnological opportunities to increase the desired beneficial properties of fungi, there is a significant lack of information regarding the potential increase in the antioxidant activity of basidiomycete mycelia under controlled conditions. This study focused on evaluating the effect of cultivation conditions on the growth of *F. pinicola* in order to enhance AOA as well as TPC under submerged static cultivation in flask.

Determining the optimal cultivation conditions, including temperature, pH, nutrient availability, and oxygen levels, is a key biotechnological approach to enhancing mycelium with specific properties, in particular AOA [65–69]. Fungal cultivation is primarily focused on energy and biomass production. For *F. pinicola*, the best cultivation conditions were found to be 25 °C, pH 2.5, with galactose, yeast extract, and a cultivation duration of 9 to 35 days under submerged shaking or static conditions, respectively. The highest TPC in *F. pinicola* mycelium was achieved at 25 °C, pH 2.5, using xylose, peptone, and a cultivation period of 5–7 or 28 days under submerged shaking or static conditions, respectively. For AOA, a more suitable temperature was 30 °C, with pH 3.5–5.0, using the same carbon and nitrogen sources, and the same cultivation period as for TPC production. Since different phenols are major contributors to AOA [41, 70, 71], some parameters that optimize phenols production also enhance AOA. This is in line with the report by Abo-Elmagd [67], which showed that the highest antioxidant potential of *Chaetomium madrasense* under different cultivation conditions, such as temperature, pH, carbon and nitrogen sources, as well as incubation period, correlates positively with the maximal phenolic content.

Based on our experimental results, some differences in optimal cultivation parameters necessary for fungal growth, total polyphenol production, and AOA acquisition in *F. pinicola* were established. Generally, this finding can be explained by the different biological and environmental requirements for each process [72]. These

processes are interrelated, but often require different conditions to achieve maximum effect. Each process has distinct optimal parameters because they reflect different metabolic states of the fungus. The optimal conditions for fungal growth focus on maximizing biomass by providing ideal cultivation conditions that support rapid and efficient cellular processes. In contrast, the optimal conditions for metabolite production which possesses biological activity often involve inducing stress responses, nutrient limitation, and specific environmental cues that trigger the synthesis of secondary metabolites.

The similarities in the optimal pH and temperature for fungal growth and TPC formation, and the differences in AOA, could be explained by the specific biochemical and physiological factors involved in each process. Productive fungal growth and polyphenol formation require the same optimal pH and temperature conditions, probably due to interrelated metabolic pathways, enzyme activity and cellular homeostasis [72]. In contrast, the values of pH and temperature reqiered for displaying of AOA of *F. pinicola* mycelium may be due to the specific stability, reactivity, and redox behavior of antioxidant compounds, as well as the need to effectively interact with ROS [73].

The significant effect of incubation period on TPC and AOA of *F. pinicola* mycelium obtained under stirring and static cultivation conditions was revealed. This can be explained by several key factors such as oxygen availability, nutrient distribution, shear stress, cell density and growth phase. Stirring accelerates these factors, leading to earlier peaks in AOA, while static conditions lead to a slower, but prolonged increase in antioxidant production.

It should be noted that a pH range from 2.5 to 5.0 contributed to the highest level of TPC and AOA of *F. pinicola* mycelium. The biosynthesis of phenolic compounds in fungi involves specific enzymes, such as phenylalanine ammonia-lyase (PAL), which is crucial in the phenylpropanoid pathway [74]. A pH range of 2.5 to 5.0 could provide the ideal conditions for these enzymes to function efficiently, leading to increased production of phenolic compounds which may be more active. The solubility and stability of phenolic compounds can be pH-dependent [75]. Acidic pH levels may contribute to higher extraction efficiency and phenols accumulation in the mycelium enhancing their ability to scavenge free radicals and exhibit AOA. Under acidic conditions, the fungus may experience increased oxidative stress, prompting the production of antioxidants as a protective measure. This could explain why both phenol levels and AOA peak within this pH range. Different antioxidant mechanisms, such as hydrogen atom transfer or single-electron transfer, can be more or less effective depending on the pH [76]. The acidic pH range might favor the specific mechanism (probably most efficient electron transfer) that *F. pinicola* uses for its antioxidant defense, leading to higher observed activity. It is known that *F. pinicola* naturally grows on coniferous wood, which can create an acidic environment due to the decomposition of organic matter and the presence of acidic compounds like tannins. The fungus might be evolutionarily adapted to thrive and produce secondary metabolites in such acidic conditions, reflecting its natural ecological niche.

The established strong correlation indicates that phenolic compounds are likely the primary contributors to the AOA within the system. A similar observation was found in another study [77]. Phenols play one of a key role in neutralising free radicals and protecting against oxidative stress [78]. In addition, the consistent correlation across different conditions might reflect an inherent biological strategy where organisms boost phenolic production as a response to environmental stress (e.g., changes in nutrient availability or pH). This also can mean, that phenols play a critical role in the organism's adaptation and survival strategies. The prospect of a strong correlation between TPC and AOA under various cultivation conditions is well-supported and presents significant opportunities for biotechnological applications. If phenol production is a key determinant of antioxidant activity, genetic engineering or selective breeding efforts can focus on enhancing phenol biosynthesis pathways. This could lead to organisms or crops with superior antioxidant properties, useful in agriculture and biotechnology. Additionally, the ability to predict AOA based on phenol content across various conditions can lead to more standardized and consistent product quality, which is crucial in commercial applications.

Producing mycelium from basidiomycetes with beneficial properties involves selecting the right strains, fine-tuning growth conditions and applying modern biotechnological methods. The ability of the studied *F. pinicola* strain to grow, produce total phenols, and exhibit antioxidant activity under all experimental conditions (except with sodium nitrite) underscores its significant biotechnological potential for producing valuable secondary metabolites. Our next research step will be focused on creating conditions that support secondary metabolite production. The results of adjusting the cultivation conditions – considering three factors: temperature, carbon source, and pH – demonstrated the effectiveness of this approach. The findings show the potential to increase phenolic compounds production by 2.25 and 2.23 times under shaking and static conditions, respectively, while maintaining a high level of activity. Final conclusions can be drawn after modeling with all factors and experimentally confirming the model's effectiveness. Dresh et al. [48] found that the optimal temperature for *F. pinicola* colony growth was strain-dependent: 25 °C was ideal for eight strains, while 32 °C was optimal for two strains.

The optimal temperature of 20 °C and the use of yeast extract as a nitrogen source for *F. pinicola* growth align with the findings of Choi et al. [12]. In those studies, glucose was identified as the optimal carbon source, and a pH range of 5.5−6.5 was suitable for *F. pinicola* biomass production. However, their study was limited to a pH range of 4.0 to 8.0 over four days, while our research encompassed a broader pH range and a longer duration. It should be noted that the same temperature and pH levels were suitable for *F. pinicola* growth and exopolysaccharide production, which aligns with our findings that certain cultivation conditions support both biomass and metabolite production.

Du et al. [47] found that the best carbon and nitrogen sources for mycelial growth of *F. pinicola* were soluble starch and yeast paste, with an ideal culture temperature of 31 °C and an optimal pH of 6.0. The optimal temperature of 30 °C and an extended static cultivation period of 20 days were found to be suitable for enhancing AOA and TPC in the mycelium of *Xylaria feejeensis* [65], which is consistent with our results. In contrast to our findings, pH 6.0, dextrose, and yeast extract supported the AOA of *X. feejeensis*, indicating the species-specific nutritional needs and physiology of this fungus.

The morphology of *F. pinicola* colonies on agar media has been presented in the literature [79]. Studying the morphology of fungal colonies in submerged culture is essential, as even minor changes in fungal morphology are well known to result in significant fluctuations in product yield, which can affect mass transfer and mixing [80]. It's also necessary to maintain a specific morphological structure in order to optimize the production of the desired metabolites [81–83]. To the best of our knowledge, this is the first report describing the growth morphology of *F. pinicola* in the form of pellets and a mycelial mat under submerged cultivation conditions. These are typical morphologies for basidiomycetes growth under shaking and static conditions, respectively. Observing these differences provides insights into how environmental factors like oxygen availability and nutrient diffusion affect fungal growth. Shaking promotes better oxygenation and uniform nutrient distribution in the culture medium [83, 84], whereas static conditions can result in oxygen limitation, especially in the deeper parts of the mycelial mat. Morphological differences in response to these conditions help in understanding how fungi adapt to changes in oxygen and nutrient availability, which is crucial for optimizing industrial fermentation processes.

Conclusion

Growth, phenols synthesis and antioxidant activity of the mycelium of *F. pinicola* are determined by the complex relationship between the genetically conditioned properties of the fungus, its adaptive abilities, and physiological mechanisms of life. The study highlights the importance of physicochemical cultivation conditions of *F. pinicola* mycelium for the production of biomass with antioxidant properties and high phenolic compounds content.

Understanding the balance between different cultivation parameters is essential to optimize the cultivation of *F. pinicola*. Controlling cultivation parameters makes it possible to regulate the metabolic and biochemical processes of the fungus, helping to achieve a balance in obtaining optimal amounts of biomass and antioxidant activity of phenols. This balance is critical for future prospects for medical and industrial applications where consistent production of bioactive compounds is required.

Acknowledgements
We would like to extend our heartfelt gratitude to Prof. Nina A. Bisko (M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine) for providing of fungi from the Culture Collection of Mushrooms (IBK).

Author contributions
T.K. conceptualization, methodology, investigations, formal analysis, writing original draft and editing, V.B. contributed in analyzing the results, writing and editing, V.D. and A.P. investigated DNK, T.Z. assisted in conducting experiments with submerged shaking cultivation, and Y.B. made contributions in work management, and edited the manuscript. All these authors have substantial contributions to the final manuscript and approved this submission.

Funding
This research was funded by the National Academy of Sciences of Ukraine, state registration number 0124U002425.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This research does not involve any ethical issues.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Plant Food Products and Biofortification, Institute of Food Biotechnology and Genomics of the National Academy of Sciences of Ukraine, 2a Baidy-Vyshnevetskoho Str., Kyiv 04123, Ukraine
[2]Department of Plant Biology, Taras Shevchenko National University of Kyiv, 64/13 Volodymyrska Str., Kyiv 01601, Ukraine
[3]Kholodnyi Yar National Nature Park, Cherkasy region, 58 Kholodnoyarska Str., Hrushkivka 20810, Ukraine
[4]Department of Genomics and Molecular Biotechnology, Institute of Food Biotechnology and Genomics of the National Academy of Sciences of Ukraine, 2a Baidy-Vyshnevetskoho Str., Kyiv 04123, Ukraine

Received: 20 August 2024 / Accepted: 28 October 2024
Published online: 07 November 2024

References

1. Anusiya G, Gowthama Prabu U, Yamini NV, Sivarajasekar N, Rambabu K, Bharath G, Banat F. A review of the therapeutic and biological effects of

edible and wild mushrooms. Bioengineered. 2021;12(2):11239–68. https://doi.org/10.1080/21655979.2021.2001183.
2. Venturella G, Ferraro V, Cirlincione F, Gargano ML. Medicinal mushrooms: Bioactive compounds, Use, and clinical trials. Int J Mol Sci. 2021;22(2):634. https://doi.org/10.3390/ijms22020634.
3. Bhambri A, Srivastava M, Mahale VG, Mahale S, Karn SK. Mushrooms as potential sources of active metabolites and Medicines. Front Microbiol. 2022;13:837266. https://doi.org/10.3389/fmicb.2022.837266.
4. Bell V, Silva CRPG, Guina J, Fernandes TH. Mushrooms as future generation healthy foods. Front Nutr. 2022;9:1050099. https://doi.org/10.3389/fnut.2022.1050099.
5. Panda SK, Luyten W. Medicinal mushrooms: clinical perspective and challenges. Drug Discov Today. 2022;27(2):636–51. https://doi.org/10.1016/j.drudis.2021.11.017.
6. Şöhretoğlu D, Kuruüzüm-Uz A. Biological activities of some Edible mushrooms. In: Agrawal DC, Dhanasekaran M, editors. Mushrooms with therapeutic potentials. Springer, Singapore. https://doi.org/10.1007/978-981-19-9550-7_14
7. Bishop KS. Characterisation of extracts and anti-cancer activities of *Fomitopsis pinicola*. Nutrients. 2020;12(3):609. https://doi.org/10.3390/nu12030609.
8. Zahid MT, Idrees M, Ying W, Zaki AH, Abdullah I, Haiying B. Review of chemical constituents and pharmacology of brownrot fungus *Fomitopsis pinicola*. J Nat Sci Res. 2020;10:58–68.
9. 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: Ethnobiology of Uzbekistan. Ethnobiology (Khojimatov OK, Gafforov Y, Bussmann RW, eds) Springer, Cham, pp. 1085–1101. https://doi.org/10.1007/978-3-031-23031-8_108
10. Grienke U, Zöll M, Peintner U, Rollinger J. European medicinal polypores – a modern view on traditional uses. J Ethnopharmacol. https://doi.org/10.1016/j.jep.2014.04.030
11. Yoshikawa K, Inoue M, Matsumoto Y, Sakakibara C, Miyataka H, Matsumoto H, Arihara S. Lanostane triterpenoids and Triterpene Glycosides from the Fruit body of *Fomitopsis pinicola* and their inhibitory activity against COX-1 and COX-2. J Nat Prod. 2005;68:69–73. https://doi.org/10.1021/np040130b.
12. Choi D, Park SS, Ding JL, et al. Effects of *Fomitopsis pinicola* extracts on antioxidant and antitumor activities. Biotechnol Bioprocess Eng. 2007;12:516–24. https://doi.org/10.1007/BF02931349.
13. Wang Y, Cheng X, Wang P, Wang L, Fan J, Wang X, Liu Q. Investigating Migration inhibition and apoptotic effects of *Fomitopsis pinicola* Chloroform Extract on Human Colorectal Cancer SW-480 cells. PLoS ONE. 2014;9:e101303. https://doi.org/10.1371/journal.pone.0101303.
14. Wu HT, Lu FH, Su YC, Ou HY, Hung HC, Wu JS, Chang CJ. In vivo and in vitro anti-tumor effects of fungal extracts. Molecules. 2014;19:2546–56. https://doi.org/10.3390/molecules19022546.
15. Gao Y, Wang P, Wang Y, Wu L, Wang X, Zhang K, Liu Q. Vitro and in vivo activity of *Fomitopsis Pinicola* (Sw. Ex Fr.) Karst Chloroform (Fpkc) Extract against S180 Tumor cells. Cell Physiol Biochem. 2017;44:2042–56. https://doi.org/10.1159/000485944.
16. Angelini P, Tirillini B, Bistocchi G, Arcangeli A, Rubini A, Pellegrino RM, Fabiani R, Cruciani G, Venanzoni R, Rosignoli P. Overview of the Biological activities of a methanol extract from Wild Red Belt Conk, *Fomitopsis pinicola* (Agaricomycetes), fruiting bodies from Central Italy. Int J Med Mushrooms. 2018;20(11):1047–63. https://doi.org/10.1615/IntJMedMushrooms.2018028595.
17. Rehman S, Jermy R, Mousa Asiri S, Shah MA, Farooq R, Ravinayagam V, Azam Ansari M, Alsalem Z, Al Jindan R, Reshi Z, Khan FA. Using *Fomitopsis pinicola* for bioinspired synthesis of titanium dioxide and silver nanoparticles, targeting biomedical applications. RSC Adv. 2020;10(53):32137–47. https://doi.org/10.1039/d0ra02637a.
18. Zhang Z, Wu C, Wang M, Chen J, Lv G. Chemical fingerprinting and the biological properties of extracts from *Fomitopsis pinicola*. Arab J Chem. 2023;16(5):104669. https://doi.org/10.1016/j.arabjc.2023.104669.
19. Kozarski M, Klaus A, Špirović-Trifunović B, Miletić S, Lazić V, Žižak Ž, Vunduk J. Bioprospecting of selected species of Polypore Fungi from the Western Balkans. Molecules. 2024;29(2):314. https://doi.org/10.3390/molecules29020314.
20. Chen G, Qian W, Li J, Xu Y, Chen K. Exopolysaccharide of Antarctic bacterium *Pseudoaltermonas* sp. S-5 induces apoptosis in K562 cells. Carbohydr Polym. 2015;121:107–14. https://doi.org/10.1016/j.carbpol.2014.12.045.
21. Peng XR, Su HG, Liu JH, Huang YJ, Yang XZ, Li ZR, Zhou L, Qiu MH. C30 and C31 triterpenoids and triterpene sugar esters with cytotoxic activities from edible mushroom *Fomitopsis pinicola* (sw. Ex Fr.) Krast. J Agric Food Chem. 2019;67(37):10330–41.
22. Pala SA, Wani AH, Ganai BA. Antimicrobial potential of some wild macromycetes collected from Kashmir Himalayas. Plant Sci Today. 2019;6(2):137–46. https://horizonepublishing.com/journals/index.php/PST/article/view/503.
23. Metreveli E, Khardziani T, Dibedulidze K, Elisashvili V. Improvement of Antibacterial Activity of Red Belt Conk Medicinal Mushroom, *Fomitopsis pinicola* BCC58 (Agaricomycetes), in fermentation of lignocellulosic materials. Int J Med Mushrooms. 2021;23(1):27–37. https://doi.org/10.1615/IntJMedMushrooms.2020037169.
24. Keller AC, Maillard MP, Hostettmann K. Antimicrobial steroids from the fungus *Fomitopsis pinicola*. Phytochemistry. 1996;41(4):1041–6.
25. Liu X-T, Winkler AL, Schwan WR, et al. Antibacterial compounds from mushrooms II: lanostane triterpenoids and an ergostane steroids with activity against *Bacillus cereus* isolated from *Fomitopsis pinicola*. Planta Med. 2010;76:464–6.
26. Krupodorova TA, Barshteyn VYu, Zabeida EF, Pokas EV. Antibacterial activity of Macromycetes mycelia and culture liquid. Microbiol Biotechnol Lett. 2016;44(3):246–53.
27. Guler P, Akata I, Kutluer F. Antifungal activities of *Fomitopsis pinicola* (Sw.:Fr) Karst. And *Lactarius Vellereus* (Pers.) Fr. Afr J Biotechnol. 2009;8:3811–3.
28. Badalyan SM, Gharibyan NG, Shnyreva AV, Shahbazyan TA. Antifungal activity of *Fomitopsis pinicola* collections against potentially pathogenic for humans and animals filamentous fungi. ICMBMP8. New Delhi, India. 2014:93 – 4.
29. Krupodorova T, Barshteyn V, Pokas O. Antagonistic effectiveness of Macromycetes against *Candida albicans* strains and *issatchenkia orientalis*. Nova Biotechnol et Chim. 2021;20(1):e760. https://doi.org/10.36547/nbc.760.
30. Krupodorova T, Barshteyn V, Kizitska T, Ratushnyak V, Blume Y. Antagonistic activity of selected macromycetes against two harmful micromycetes. Czech Mycol. 2023;75(1):85–100.
31. Tai SH, Kuo PC, Hung CC, Lin YH, Hwang TL, Lam SH, Kuo DH, Wu JB, Hung HY, Wu TS. Bioassay-guided purification of sesquiterpenoids from the fruiting bodies of *Fomitopsis pinicola* and their anti-inflammatory activity. RSC Adv. 2019;9(59):34184–95.
32. Lee SI, Kim JS, Oh SH, Park KY, Lee HG, Kim SD. Antihyperglycemic effect of *Fomitopsis pinicola* extracts in streptozotocin-induced diabetic rats. J Med Food. 2008;11(3):518–24.
33. Cha WS, Ding JL, Shin HJ, Kim JS, Kim YS, Choi DB, et al. Effect of *Fomitopsis pinicola* extract on blood glucose and lipid metabolism in diabetic rats. Korean J Chem Eng. 2009;26(6):1696–9.
34. Abo Nahas HH, Darwish AMG, Abo Nahas YH, Elsayed MA, Abdel-Azeem AM, Abdel-Azeem MA et al. Fungi as a Gold Mine of Antioxidants. Chapter 2. In: Abdel-Azeem A.M. editors, Industrially Important Fungi for Sustainable Development. Springer Nature. Switzerland AG. 2021. https://doi.org/10.1007/978-3-030-85603-8_2
35. Hao L, Sheng Z, Lu J, Tao R, Jia S. Characterization and antioxidant activities of extracellular and intracellular polysaccharides from *Fomitopsis pinicola*. Carbohydr Polym. 2016;141:54–9.
36. Onar O, Akata I, Sağdiçoğlu Celep G, Yildirim Ö. Antioxidant activity of extracts from the Red-Belt Conk Medicinal mushroom, *Fomitopsis pinicola* (Agaricomycetes), and its Modulatory effects on antioxidant enzymes. Int J Med Mushrooms. 2016;18(6):501–8.
37. Sevindik M, Akgul H, Akata I, Alli H, Selamoglu Z. The benefits of natural antioxidants make the need to develop useful strategies to improve their synthesis. Acta Aliment. 2017;46(4):464–9. https://doi.org/10.1556/066.2017.46.4.9.
38. Nie L, Hao L, Wang T, Liu Y, Zhang L, Lu J, et al. Antioxidant activity of crude polysaccharides from *Fomitopsis pinicola* from different Geographical origins. J Food Sci. 2019;40(19):60–8.
39. Blois MS. Antioxidant determinations by the Use of a stable free Radical. Nature. 1958;181(4617):1199–200. https://doi.org/10.1038/1811199a0.
40. Gulcin I, Alwasel SH. DPPH Radical Scavenging Assay. Processes. 2023;11:2248. https://doi.org/10.3390/pr11082248.
41. Martinez-Burgos WJ, Montes Montes E, Pozzan R, Serra JL, Torres DO, Manzoki MC, Vieira RL, dos Reis GA, Rodrigues C, Karp SG, et al. Bioactive compounds produced by macromycetes for application in the Pharmaceutical Sector: patents and products. Fermentation. 2024;10:275. https://doi.org/10.3390/fermentation10060275.
42. Folin O, Ciocalteau V. Tyrosine and tryptophane in proteins. J Biol Chem. 1927;73(2):627–48.
43. Singleton VL, Rossi JL. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. Am J Enol Viticult. 1965;16:144–58.

44. Lamuela-Raventós RM. Folin-Ciocalteu method for the measurement of total phenolic content and antioxidant capacity. Meas Antioxid Activity Capacity. 2017;107–15. https://doi.org/10.1002/9781119135388.ch6.
45. Sulkowska-Ziaja K, Muszynska B, Motyl P, Pasko P, Ekiert H. Phenolic compounds and antioxidant activity in some species of polyporoid mushrooms from Poland. Int J Med Mushrooms. 2012;14(4):385–93. https://doi.org/10.1615/intjmedmushr.v14.i4.60.
46. Choi D, Maeng JM, Ding JL, Cha WS. Exopolysaccharide production and mycelial growth in an air-lift bioreactor using *Fomitopsis pinicola*. J Microbiol Biotechnol. 2007;17(8):1369–78.
47. Du P, Cao TX, Zhang LL, Huang YQ, Chen JZ. Cultivation and Medicinal Value of the Red Belt Conk Mushroom *Fomitopsis pinicola* (Agaricomycetes). Int J Med Mushrooms. 2020;22(10):1021–31. https://doi.org/10.1615/IntJMedMushrooms.2020035811.
48. Dresch P, D´Aguanno MN, Rosam K, et al. Fungal strain matters: colony growth and bioactivity of the European medicinal polypores *Fomes Fomentarius*, *Fomitopsis pinicola* and *Piptoporus Betulinus*. AMB Expr. 2015;5:4. https://doi.org/10.1186/s13568-014-0093-0.
49. Gu JM, Park SS. Optimization of endoglucanase production from *Fomitopsis pinicola* Mycelia. Korean J Microbiol Biotechnol. 2013;14(3):145–52. https://doi.org/10.4014/kjmb.1301.01007.
50. Bisko N, Lomberg M, Mykhaylova O, Mytropolska N. IBK Mushroom Culture Collection. Version 1.2., The IBK Mushroom Culture Collection of the M.G. Kholodny Institute of Botany, Kyiv, 2020; Occurrence dataset https://doi.org/10.15468/dzdsqu
51. Dellaporta S. Plant DNA miniprep and microprep: versions 2.1–2.3. In: Freeling M, Kirst G. O., editor. The Maize Handbook. New York: Springer-; 1994. pp. 522–5.
52. White TJ, Bruns T, Lee SJWT, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols: Guide Methods Appl. 1990;18(1):315–22. https://doi.org/10.1016/B978-0-12-372180-8.50042-1.
53. 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.
54. Hadi P, Markad P. The role of antioxidants in disease prevention: a review. Int J Health Sci. 2023;6(S8):7023–31. https://doi.org/10.53730/ijhs.v6nS8.14124.
55. Kıran TR, Otlu O, Karabulut AB. Oxidative stress and antioxidants in health and disease. J Lab Med. 2023;47(1):1–11. https://doi.org/10.1515/labmed-2022-0108.
56. Masaki H. Role of antioxidants in the skin: anti-aging effects. J Dermatol Sci. 2010;58(2):85–90. https://doi.org/10.1016/j.jdermsci.2010.03.003.
57. Rusu ME, Fizeşan I, Vlase L, Popa DS. Antioxidants in Age-Related diseases and anti-aging strategies. Antioxid (Basel). 2022;11(10):1868. https://doi.org/10.3390/antiox11101868.
58. Xu X, Liu A, Hu S, Ares I, Martínez-Larrañaga MR, Wang X, et al. Synthetic phenolic antioxidants: metabolism, hazards and mechanism of action. Food Chem. 2021;353:129488. https://doi.org/10.1016/j.foodchem.2021.129488.
59. Wang W, Xiong P, Zhang H, Zhu Q, Liao C, Jiang G. Analysis, occurrence, toxicity and environmental health risks of synthetic phenolic antioxidants: a review. Environ Res. 2021;201:111531. https://doi.org/10.1016/j.envres.2021.111531.
60. Sgroi F, Moscato CM, Moscato R. Consumer preferences for the Mediterranean Diet: results of an empirical analysis. J Agric Food Res. 2022;10:100371. https://doi.org/10.1016/j.jafr.2022.100371.
61. Asatiani MD, Elisashvili V, Songulashvili G, Reznick AZ, Wasser SP. Higher Basidiomycetes mushrooms as a source of antioxidants. In: Rai M, Kövics G, editors. Progress in Mycology. Dordrecht: Springer; 2010. https://doi.org/10.1007/978-90-481-3713-8_11.
62. Mattila H, Österman-Udd J, Mali T, Lundell T, Basidiomycota Fungi. Genomic perspective on key enzymes involved in generation and mitigation of reactive oxygen species. Front Fungal Biol. 2022;3:837605. https://doi.org/10.3389/ffunb.2022.837605.
63. Niego AG, Rapior S, Thongklang N, Raspé O, Jaidee W, Lumyong S, Hyde KD. Macrofungi as a nutraceutical source: promising Bioactive compounds and Market Value. J Fungi (Basel). 2021;7(5):397. https://doi.org/10.3390/jof7050397.
64. Bakratsas G, Polydera A, Katapodis P, Stamatis H. Recent trends in submerged cultivation of mushrooms and their application as a source of nutraceuticals and food additives. Future Foods. 2021;4:100086. https://doi.org/10.1016/j.fufo.2021.100086.
65. Rebbapragada DP, Rajagopal K. Evaluation and optimization of antioxidant potentiality of *Xylaria feejeensis* HMJAU22039. Asian J Pharm Clin Res. 2016;9(2):269–73.
66. Arora DS, Chandra P, Kau GJ. Optimization and assay of antioxidant potential of two Penicillium Spp. By different procedures. Curr Biotechnol. 2012;1(1):2–10.
67. Abo-Elmagd HI. Evaluation and optimization of antioxidant potentiality of *Chaetomium madrasense* AUMC 9376. J Genet Eng Biotechnol. 2014;12(1):21–6.
68. Gazi MR, Kanda K, Kato F. Optimization of various cultural conditions on growth and antioxidant activity aeneration by Saccharomyces cerevisiae IFO 2373. J Biol Sci. 2004;4(2):224–28.
69. Azieana J, Zainon MN, Noriham A, Rohana MN. Total phenolic and flavonoid content and antioxidant activities of ten Malaysian wild mushrooms. Open Access Libr J. 2017;4:e3987. https://doi.org/10.4236/oalib.1103987.
70. Bristy AT, Islam T, Ahmed R, Hossain J, Reza HM, Jain P. Evaluation of Total Phenolic Content, HPLC Analysis, and antioxidant potential of three local varieties of mushroom: a comparative study. Int J Food Sci. 2022;3834936. https://doi.org/10.1155/2022/3834936.
71. Gonzalez JM, Aranda B. Microbial Growth under limiting conditions-future perspectives. Microorganisms. 2023;11(7):1641. https://doi.org/10.3390/microorganisms11071641.
72. Añazco C, Riedelsberger J, Vega-Montoto L, Rojas A. Exploring the interplay between polyphenols and Lysyl Oxidase Enzymes for maintaining Extracellular Matrix Homeostasis. Int J Mol Sci. 2023;24:10985. https://doi.org/10.3390/ijms241310985.
73. Hunyadi A. The mechanism(s) of action of antioxidants: from scavenging reactive oxygen/nitrogen species to redox signaling and the generation of bioactive secondary metabolites. Med Res Rev. 2019;39(6):2505–33. https://doi.org/10.1002/med.21592.
74. Hyun MW, Yun YH, Kim JY, Kim SH. Fungal and Plant Phenylalanine Ammonia-lyase. Mycobiology. 2011;39(4):257–65. https://doi.org/10.5941/MYCO.2011.39.4.257.
75. Friedman M, Jürgens HS. Effect of pH on the stability of plant phenolic compounds. J Agric Food Chem. 2000;48(6):2101–10. https://doi.org/10.1021/jf990489j.
76. Xie Y, Li X, Chen J, Deng Y, Lu W, Chen D. pH Effect and Chemical mechanisms of antioxidant higenamine. Molecules. 2018;23(9):2176. https://doi.org/10.3390/molecules23092176.
77. Atamanchuk AR, Bisko NA. Dynamics of the phenolic constituents and antioxidant activity in submerged cultures of *Xylaria* species. Biotechnologia Acta. 2023;16(6):82–7.
78. Rudrapal M, Khairnar SJ, Khan J, Dukhyil AB, Ansari MA, Alomary MN, Alshabrmi FM, Palai S, Deb PK, Devi R. Dietary polyphenols and their role in oxidative stress-Induced Human diseases: insights into Protective effects, antioxidant potentials and mechanism(s) of action. Front Pharmacol. 2022;13:806470. https://doi.org/10.3389/fphar.2022.806470.
79. Purnomo AS, Sariwati A, Kamei I. Synergistic interaction of a consortium of the brown-rot fungus *Fomitopsis pinicola* and the bacterium *Ralstonia pickettii* for DDT biodegradation. Heliyon. 2020;6(6):e04027. https://doi.org/10.1016/j.heliyon.2020.e04027.
80. El Enshasy HA. Fungal morphology: a challenge in bioprocess engineering industries for product development. Curr Opin Cheml Eng. 2022;35:100729. https://doi.org/10.1016/j.coche.2021.100729.
81. Braun S, Vecht-Lifshitz SE. Mycelial morphology and metabolite production. Trends Biotechnol. 1991;9(2):63–8. https://doi.org/10.1016/0167-7799(91)90191-j.
82. Hamedi A, Ghanati F, Vahidi H. Study on the effects of different culture conditions on the morphology of *Agaricus Blazei* and the relationship between morphology and biomass or EPS production. Ann Microbiol. 2012;62:699–707. https://doi.org/10.1007/s13213-011-0309-3.
83. Lueangjaroenkit P, Teerapatsakul C, Chitradon L. Morphological characteristic regulation of ligninolytic enzyme produced by *trametes polyzona*. Mycobiology. 2018;46(4):396–406. https://doi.org/10.1080/12298093.2018.1537586.
84. Büchs J. Introduction to advantages and problems of shaken cultures. Biochem Eng J. 2001;7(2):91–8. https://doi.org/10.1016/s1369-703x(00)00106-6.

Publisher's note