ANALYSIS OF GROWTH CONDITIONS OF THE GENUS HERICIUM AS A POSSIBLE TOOL FOR SUSTAINABLE BIORESOURCE MANAGEMENT

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The article is dedicated to reviewing the potential of fungi of the genus Hericium in the context of sustainable development. Both theoretical and practical aspects of the application of these basidiomycetes are discussed. Particular attention is given to the possibilities of using these fungi in the field of healthcare, with a special focus on their neuroprotective, antioxidant, antimicrobial, anti-inflammatory, and other therapeutic properties. The article also examines cultivation strategies, nutrient medium selection, and optimal growth conditions that can ensure maximum yields of biologically active compounds with minimal environmental impact. The results of studies on the growth rates of Hericium strains in various nutrient media, including those containing cellulose-based components, are presented. It is shown that the most favorable media for achieving maximum growth characteristics of the studied fungal strains were malt agar and malt agar with the addition of cellulose-containing supplements: oak bark and fir needles, where the maximum growth rates were 5.5-5.7 mm/day.

Keywords: mushroom, media, fruiting bodies, growth rate, in vitro

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Introduction

In today's world, where global challenges such as climate change, healthcare, and food security require innovative solutions, biotechnology plays a vital role in achieving the Sustainable Development Goals (SDGs). One promising direction in this field is the study of fungi of the genus *Hericium*, which combine nutritional and medicinal value with opportunities for sustainable production. These mushrooms have been used in traditional Eastern medicine for a long time, but they are now finding new applications in modern biotechnological research. As functional food products, mushrooms provide high nutritional value and are a valuable source of new therapeutic compounds (Chong, Fung, Wong, & Lim, 2019), which can contribute to the realization of SDG 3, "Good Health and Well-being".

The genus *Hericium*, which belongs to the family *Hericiaceae*, includes species that are both edible and medicinal. One of the most well-known representatives of this genus is *Hericium erinaceus* (lion's mane mushroom). This edible mushroom has been used for over 2000 years in Chinese medicine to treat gastrointestinal diseases and nervous system disorders (Sokol, Golak-Siwulska, Sobieralski, Siwulski, & Górka, 2015; Lomberg, 2021). Recent studies have confirmed its neuroprotective properties, making it a promising candidate for the development of drugs for the

treatment of neurodegenerative diseases such as Alzheimer's disease. This aligns with SDG 3, supporting the development of new therapeutic approaches to promote the health and well-being of the population.

In addition to medical applications, the cultivation of *H. erinaceus* opens up opportunities for realizing SDG 12, "Responsible Consumption and Production," and SDG 15, "Life on Land." This mushroom belongs to wood-decaying species, which allows the use of cellulose-containing waste, such as sawdust, straw, or other agricultural residues, as a substrate for its growth. This approach promotes the disposal of bio-waste and reduces their accumulation in landfills, thus decreasing the negative impact on the environment. Implementing such practices in industrial production aligns with the principles of the circular economy, enhancing resource efficiency and contributing to sustainable development.

The fruiting bodies of *H. erinaceus* develop singly or in small fleshy clusters, characterized by a hymenophore with structures forming thin, downward-hanging spines of varying lengths, resembling a hedgehog (Gonkhom et al., 2022).

Mushrooms of the genus *Hericium* are an excellent source of new therapeutic compounds that have unique effects on the nervous system, digestive organs, brain, and other human organs and systems. In particular, metabolites of *H. erinaceus* have been extensively studied and demonstrated antitumor activity, immune activation, cholesterol reduction, and stimulation of neurite growth, as well as effectiveness in treating depressive disorders and certain neurological diseases (such as Alzheimer's and Parkinson's) (Gonkhom et al., 2021; Lomberg, 2021).

Research results indicate that both fruiting bodies and mycelium, as well as the cultural liquid of *Hericium* fungi, can be sources of fungal polysaccharides. Specifically, the possibility of obtaining polysaccharides from the fruiting bodies of *H. erinaceus* has been demonstrated. These polysaccharides, at inhibitory concentrations of 0.5 to 4 mg/mL, impeded the proliferation of cell lines by inducing cell cycle arrest, which may have potential anticancer effects (Wang et al., 2015; Yang et al., 2021). Another species, *H. coralloides*, when grown in a medium including maltose, soy peptone, and yeast extract, was able to produce up to 0.13 g/L of extracellular polysaccharides (EPS). The obtained EPS exhibited significant toxicity against the Caucasian adenocarcinoma cell line of the human stomach (Tabibzadeh et al., 2022). Moreover, polysaccharides from *Hericium* fungi are capable of regulating immunity by stimulating the phagocytic activity of macrophages (Yang et al., 2021).

Polysaccharides from *H. erinaceus* have attracted researchers' attention as a potential means of regulating the species composition of gut microbiota. Studies on the digestibility of fungal polysaccharides and their effects on digestive processes have shown a significant impact of fungal exopolysaccharides, particularly the polysaccharide HEP-50, on increasing the relative concentration of fatty acid-producing bacteria: *Bifidobacterium*, *Faecalibacterium*, *Blautia*, *Butyricicoccus*, and *Lactobacillus*. Additionally, the studied polysaccharides exhibited an inhibitory effect against opportunistic pathogenic bacteria: *Escherichia*, *Shigella*, *Klebsiella*, and *Enterobacter* (Tian et al., 2022). This highlights the potential for using such fungal metabolites as prebiotics for the production of functional food products.

In the literature, there is a substantial body of information regarding the antimicrobial properties of low-molecular-weight compounds synthesized by the mushroom *H. erinaceus*, specifically erinacines (Cheng, et al., 2021; Wolters et al., 2015; Chang et al., 2016; Li et al., 2019;

Ratto et al., 2019; Rupcic et al., 2018), hericenones (Ratto et al., 2019; Ruan et al., 2023), and corallocins but from *H. coralloides* (Wittstein et al., 2016).

The conditions for cultivating mushrooms have a decisive influence on the final concentration of practically valuable metabolites. For instance, when growing the *H. erinaceus* MU30296 strain in a liquid medium with the following composition (g/L): glucose – 70, NH_4NO_3 – 8, NaCl – 1.45, $ZnSO_4 \cdot 7H_2O$ – 0.055, and KH_2PO_4 – 1.0 for 12 days, the maximum concentration of the terpenoid erinacine A was 56.8 mg/mL, with a specific concentration of 12.85 mg/g dry biomass. Under solid-state fermentation of this strain on six different media based on grain masses such as polished rice, corn, adlay grain, brown rice, red beans, and sesame over 20 days, a maximum concentration of erinacine A of 8.3 g/kg substrate and a specific concentration of 165.36 mg/g dry biomass were achieved (Cheng et al., 2021).

An optimized approach to obtaining high concentrations of erinacine C using the *H. erinaceus* FU70034 strain involved a combination of preliminary biomass production on a casein-peptone medium followed by deep cultivation of the strain on a medium containing 36 g/L of glucose as a carbon source, 5 g/L of oat flakes, and 0.5 g/L of Edamin® K as complex nitrogen sources, 0.5 g/L of ammonium sulfate as an inorganic nitrogen source, 1.5 g/L of calcium carbonate, and 100 mM of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid to maintain an optimal pH level of 7.5 during cultivation. Under these conditions, a maximum concentration of 2.73 g/L of erinacine C was achieved (Wolters et al., 2015).

Cultivation of *Hericium* mushrooms to obtain metabolites with antimicrobial and other therapeutic properties is scalable for industrial production. Erinacine at a concentration of 225±54 mg/L was obtained during the cultivation of the *H. erinaceus* BCRC 35669 strain in a 500 L bioreactor. The process lasted for 8 days at a temperature of 28 °C, with a constant low stirring speed of 30 RPM and an aeration intensity of 50 L/L·min. The obtained erinacine demonstrated the ability to prevent apoptosis and stimulate the proliferation of PC-12 cell lines. Furthermore, it exhibited protective properties, supporting cell viability in media containing 20 mM glutamate (Cheng et al., 2021).

Compounds isolated from the fruiting bodies and mycelium of *Hericium* mushrooms have a pronounced neurotrophic effect and can influence human nerve cells, alleviating symptoms of neurodegenerative diseases such as Alzheimer's and Parkinson's disease (Gonkhom et al., 2021).

The cultivation process of the *H. erinaceus* BCRC 35669 strain was scaled up from a 2-liter flask to fermenters with volumes of 500 L and 20 m^3. The duration of cultivation was 5 and 12 days, respectively. In the basidioma of *H. erinaceus*, concentrations of erinacine A, as well as hericenones C and D, were found to be 250 µg/g, 500 µg/g, and less than 20 µg/g, respectively. These compounds demonstrated neurostimulating effects in experimental mice of various age groups (Li et al., 2019).

Mushrooms of the genus *H. erinaceus* are a source of natural biologically active compounds, such as hericenones O, P, Q, and R, which are typically extracted from the fruiting bodies of these fungi. Assessment of biological activity has shown that hericenone Q exhibits significant cytotoxic activity against Hep-G2 (human hepatocellular carcinoma cell line) with IC50 values of 23.89 µM and against HCT-116 (human colon cancer cell line) with IC50 values of 65.64 µM (Ruan et al., 2023). Hericenones C, D, and E stimulate the synthesis of nerve growth factor (NGF). It has been reported that hericenone F is responsible for the anti-inflammatory effect by reducing the release of nitric oxide (Corana et al., 2019).

Since the chemical composition of fungal biomass largely depends on the growing environment and cultivation conditions, and natural populations of *Hericium* fungi are limited, research on methods for their artificial cultivation is essential. The high demand for these mushrooms in industry and pharmaceuticals necessitates the development of effective biotechnological solutions that facilitate the sustainable production of valuable biological compounds. Additionally, an important research direction is the evaluation of their growth conditions on various nutrient media, including cellulose-containing substrates, the utilization of which is also a crucial practical task for achieving ecological and economic goals of sustainable development.

Materials and Methods

Mushroom Strains

The objects of the research were pure cultures of basidiomycete fungi of various geographical origins, preserved in the IBK Mushroom Culture Collection at the M.G. Kholodny Institute of Botany, NAS of Ukraine. Ten strains of *H. erinaceus* were studied: 963, 965, 991, 992, 993, 1606, 1706, 1866, 2016, and 2239, as well as strains of H. abietis 2376, H. alpestre 2407, *H. cirrhatum* 2393, and *H. coralloides* 2332 (Bisko et al., 2024).

Nutrient Agar Media

The study of the morphological characteristics and growth rate of the investigated mushroom strains was conducted in Petri dishes using ten standard and modified agar nutrient media with the addition of cellulose-containing components of different compositions: natural – wort agar (8° Balling) (WA), Wort agar supplemented with 3% oak sawdust (WAO), Wort agar supplemented with 5% fir needles (WAN), oatmeal agar (OA), and wheat meal agar (WMA); and complex – malt extract agar (MEA), malt extract-peptone agar (MPA), malt extract-peptone-yeast agar (MYPA), potato-dextrose agar (PDA), and glucose-peptone-yeast agar (GPYA). All media were prepared and sterilized according to generally accepted methods (Lomberg, Solomko, 2012).

Cultural and Morphological Investigations

The fungal cultures stored in the IBK collection at a temperature of +4°C were subcultured into tubes containing WA. These tubes were used as working cultures and incubated in a thermostat at a temperature of 26±1°C for 7–10 days. Agar pieces with mycelium were used to inoculate prepared Petri dishes with WA and incubated for 10 days. Then, 5 mm diameter disks of mycelium from actively growing colonies (5–10 mm from the edge) were cut with a sterile steel tube and transferred to the center of the Petri dishes containing the aforementioned media. The calculation of the radial growth rate was performed according to the described methodology (Lomberg, Solomko, 2012). To obtain the generative stage of the fungus, after full colonization of the agar plates by mycelium, the Petri dishes were placed under daylight conditions.

The microstructures of the vegetative mycelium were examined using light microscopy. The macromorphological characteristics included a description of mycelial colonies (colony and reverse color, density, zonation, etc.). The cultivation of cultures occurred at a temperature of 26±1°C.

Results and Discussion

Microstructures and Teleomorph Stage

It has been shown that all studied strains of the species *H. erinaceus* were characterized by the presence of micromorphological features, such as numerous clamps, anastomoses, cubic and rectangular crystals, which have been previously described for this species (Buchalo et al., 2009).

It should be noted that all studied species and strains formed the teleomorph stage on various media, which serves as a reliable criterion for their identification in culture. Thus, the fruiting of strains of *H. coralloides*, *H. cirrhatum*, and *H. abietis* on wort agar is represented in Figure 1.

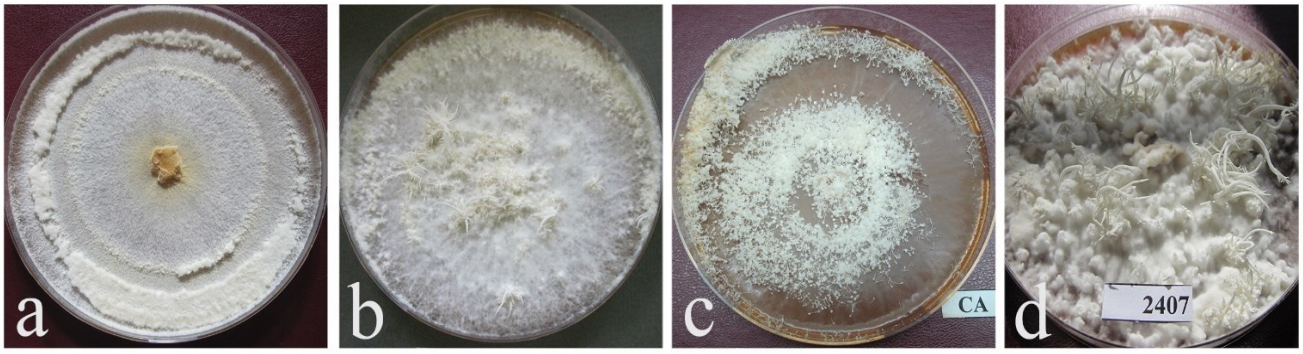

Figure 1. Growth and fruiting bodies formation of some *Hericium* species on Wort agar (WA):
a – *Hericium cirrhatum* 2393, b – *Hericium coralloides* 2332, c – *Hericium abietis* 2376, d – *Hericium flagellum* 2407

At the same time, strains of *H. erinaceus* actively formated primordia and fruiting bodies on the vast majority of nutrient media (Figure 2).

Growth Rate and Morphology Study

Given the importance of selecting biological agents capable of growing on inexpensive nutrient media, the most interesting were those studied strains of fungi that exhibited the highest radial growth rates on media containing cellulose components. Overall, the studied strains of the genus *Hericium* grew at an average growth rate ranging from 2 to 5.7 mm/day, depending on the strain and nutrient medium (Figure 3).

The morphology and density of the colonies also varied. On all studied media, whitish colonies formed, which gradually acquired cream or brown coloration over time. The reverse side of the colonies also turned yellow or yellow-brown on all media with time. The most selective media were found to be malt agar and CA supplemented with decoctions of oak bark and fir. On these media, the strains grew at maximum growth rates, and we observed the densest woolly colonies, while in some slower-growing strains, the colonies were silky with mycelial strands. The first fruiting bodies appeared on these media starting from the 20th day of cultivation. Less dense, more silky colonies formed on MPA, MYPA, GPYA, and PDA, but all strains exhibited the formation of teleomorphs. In contrast, on MEA, OA, and WMA, cultures grew significantly slower, producing thin silky colonies, with primordia and fruiting bodies either forming with a delay or being absent altogether.

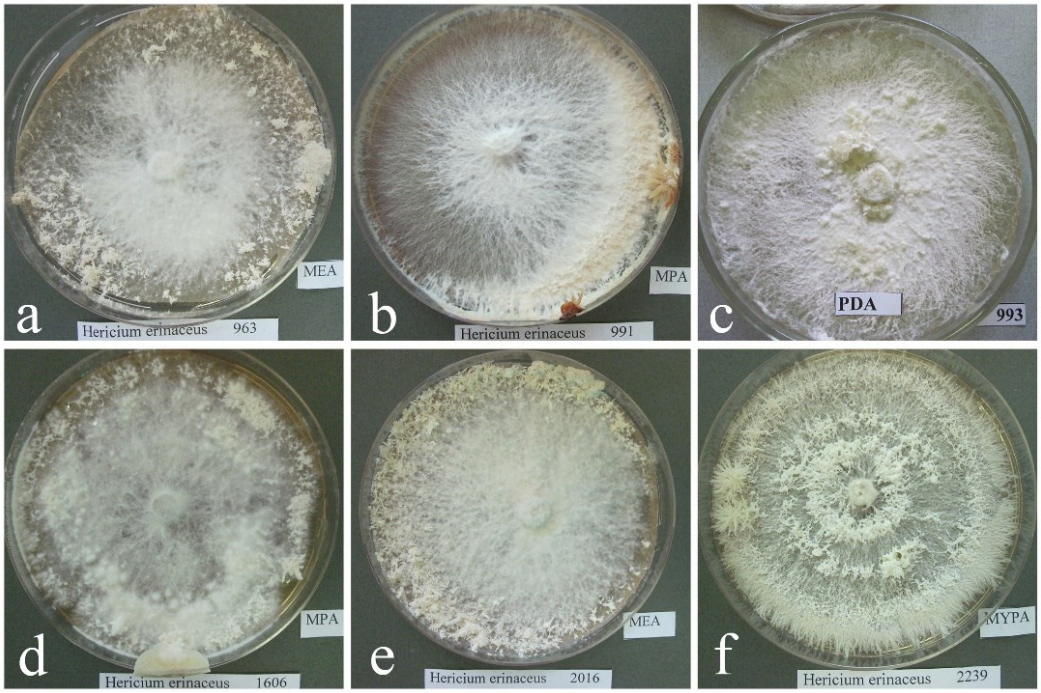

Figure 2. Growth and fruiting bodies formation of *Hericium erinaceus* strains on various agar media. The IBK strains: a – 963 on MEA, b – 991 on MPA, c – 993 on PDA, d – 1606 on MPA, e – 2016 on MEA, f – 2239 on MYPA

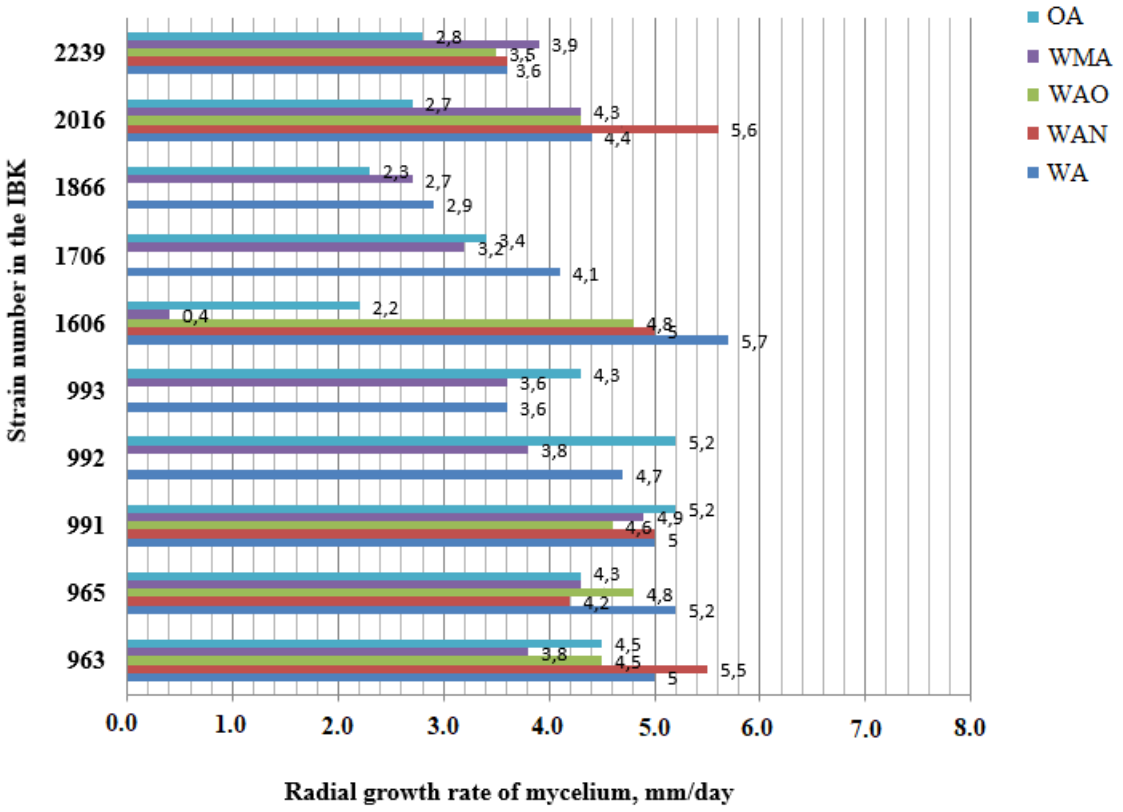

Figure 3. Radial growth rate of mycelium of *Hericium erinaceus* strains on agarized nutrient media at a temperature of 26°C

Among the studied cultures, the highest growth rate was recorded in *H. erinaceus* 1606 on OA (5.7±0.5 mm/day), followed by *H. erinaceus* 2016 and 963 on WAN (5.6±0.5 mm/day and 5.5±0.4 mm/day, respectively). Oatmeal agar also proved to be a selective medium for other strains of this species, but the white silky mycelial colonies with strands formed on it were not dense, while the densest woolly colonies were formed on wort agar with the addition of sawdust and fir. Strains 963, 991, 2016, and 1606, which grew at maximum speed on the vast majority of cellulose-containing nutrient media, showed themselves to be the most promising for further research. The slowest growth rates were observed in *H. abietis* and *H. alpestre* species. Both species exhibited a prolonged lag phase, lasting at least a week. The strain *H. abietis* 2376 grew better, achieving a growth rate of 3.7±1.0 mm/day on the selected selective medium WMA, but the colony was not dense, and no fruiting bodies formed on this medium. The best medium for cultivating *H. alpestre* 2407 proved to be WA, with a maximum growth rate of 2.3±0.3 mm/day and a minimum lag phase of 7 days.

Conclusions

Current literature indicates that the mycelium and basidiomes of *Hericium* fungi are sources of important nutrients and biologically active compounds that possess significant therapeutic potential. These fungi exhibit numerous unique properties, including antioxidant, anti-diabetic, anti-hyperglycemic, hypolipidemic, anti-inflammatory, antimicrobial, antiviral, hepatoprotective, and potential anticancer activities, making them a promising raw material for the production of specialized supplements for dietary and therapeutic nutrition. Selective media for cultivating vegetative mycelium were determined for each studied species of *Hericium*, and strains that grew at maximum growth rates on the majority of cellulose-containing nutrient media and readily transitioned to the generative growth stage were selected. The proposed strains are promising for further research and the development of biotechnologies for their cultivation, aimed at achieving Sustainable Development Goals related to improving food quality (SDG 2) and ensuring healthy lives (SDG 3).

Conflict of interest

The authors state no conflict of interest.

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