Research Article

Zhanar Narmuratova, Nina Bisko, Kairat Mustafin, Galeb Al-Maali, Alina Kerner, Svitlana Bondaruk, Zhanara Suleimenova, Aigul Kalieva, Nurlan Akhmetsadykov, Aigerim Zhakipbekova and Margarita Lomberg*

Screening of medicinal mushroom strains with antimicrobial activity and polysaccharides production

https://doi.org/10.1515/tjb-2022-0235
Received October 14, 2022; accepted March 7, 2023;
published online April 24, 2023

Abstract

Objectives: This study aimed to determine the antimicrobial activity of culture fluid and mycelial mass of selected *Hericium* strains from the IBK Mushroom Culture Collection (Kyiv, Ukraine) against bacteria and fungi, as well as to evaluate the potential of these mushrooms for biomass and extracellular polysaccharides production.

Methods: The antimicrobial activity of ethyl acetate extracts of culture fluid and aqueous-ethyl extracts of mycelial mass for three *Hericium* species: *Hericium cirrhatum* IBK 2393, *Hericium coralloides* IBK 2332, and *Hericium erinaceus* IBK 2530 against Gram-positive *Bacillus subtilis*, *Staphylococcus aureus*, and Gram-negative *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aureginosa* bacteria as well as *Aspergillus niger*, *Penicillium polonicum*, and *Mucor globosus* fungi by the disk diffusion method was conducted. Analysis of the minimum inhibitory concentration was carried out according to the broth dilution method.

Results: High antimicrobial activity of culture fluid extracts of *Hericium* mushroom species against *S. aureus* was established after 14–21 days of cultivation. No effects of *Hericium* extracts against *A. niger*, *P. polonicum*, and *M. globosus* were observed. On the 7th day of submerged cultivation, the studied strains produced the maximum yield of extracellular polysaccharides. The most promising extracellular polysaccharide (EPS) producers were strains of *H. coralloides* IBK 2332 (1.89 g/L) and *H. erinaceus* IBK 2530 (1.90 g/L). The maximal value of biomass was produced by *H. cirrhatum* IBK 2393 on the 21st day of submerged cultivation (17.45 g/L).

Conclusions: *Hericium* mushrooms have significant potential for the development of new drugs with health benefits properties.

Keywords: basidiomycetes; culture fluid; extracellular polysaccharide (EPS); *Hericium*; minimum inhibitory concentration (MIC); mycelium.

***Corresponding author: Margarita Lomberg**, Department of Mycology, M.G. Kholodny Institute of Botany of NAS of Ukraine, 2, Tereshchenkivska St., Kyiv, Ukraine, Phone: +38 (096) 524 19 196,
E-mail: margarita@lomberg.kiev.ua. https://orcid.org/0000-0001-7036-7850

Zhanar Narmuratova, Kairat Mustafin, Zhanara Suleimenova, Nurlan Akhmetsadykov and Aigerim Zhakipbekova, Department of Biochemistry, Research and Production Enterprise Antigen Co. Ltd., Almaty region, Kazakhstan. https://orcid.org/0000-0003-1641-4753 (Z. Narmuratova). https://orcid.org/0000-0001-9471-7333 (K. Mustafin). https://orcid.org/0000-0002-6524-4423 (Z. Suleimenova). https://orcid.org/0000-0001-6076-7164 (N. Akhmetsadykov). https://orcid.org/0000-0002-7927-4738 (A. Zhakipbekova)

Nina Bisko and Galeb Al-Maali, Department of Mycology, M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine, Kyiv, Ukraine. https://orcid.org/0000-0003-1894-0896 (N. Bisko). https://orcid.org/0000-0002-7489-6466 (G. Al-Maali)

Alina Kerner and Svitlana Bondaruk, National University of Food Technologies, Kyiv, Ukraine. https://orcid.org/0000-0003-2810-906X (A. Kerner). https://orcid.org/0000-0002-2052-8641 (S. Bondaruk)

Aigul Kalieva, K. Zhubanov Aktobe Regional University, Aktobe, Kazakhstan. https://orcid.org/0000-0003-1178-0236

Introduction

Edible mushrooms have not only great nutritional value and taste but also various medicinal properties [1, 2]. Most basidiomycetes contain biologically active substances with antibacterial effects, such as polysaccharides, carotenoids, phenolic compounds, terpenoids, etc., in their fruiting bodies, mycelium, and culture fluid [3, 4].

One of the priority areas in biotechnology and experimental mycology is the search for new producers of biologically active substances from edible mushrooms of

different ecological and trophic groups. One of the well-known edible mushrooms with medicinal properties confirmed by clinical studies is *Hericium erinaceus* (Bull.: Fr.) Pers. This fungus has been used in folk medicine, mainly in South-East Asia countries (China, Japan). Purified bioactive metabolites from the fruiting bodies or mycelium of *H. erinaceus* have biological activities such as antitumor, neuro- and gastroprotective, immunomodulatory, neurotrophic, antioxidant, antibacterial, hypoglycemic, and hepatoprotective [5, 6]. Among biologically active substances isolated from *Hericium* fungi are hericenones, erinacins, extracellular and endopolysaccharides, phenols, sterols, and others [2, 7]. From the moment of isolation of oncostatic polysaccharides from the fruiting bodies of some basidiomycetes, an active study of these compounds is being begun, as well as the search for their producers [8–10]. Polysaccharides obtained not only from fruiting bodies but also from submerged mycelium and culture fluid of *Hericium* fungi attracted much attention due to their strong pharmacological effect [11–14].

At present, among *Hericium* fungi, *H. erinaceus* is the most studied. At the same time, there is little information about the secondary metabolites produced by other species of *Hericium* mushrooms. Only data about erinacine isolation from *H. flagellum* and corallocins from *Hericium coralloides* which are potential therapeutic agents for neurodegenerative disorders were reported [7, 15].

Antimicrobial properties of macromycetes, including *Hericium* fungi, have been studied in numerous works [16–20]. It was found that aqueous, ethanol, methanol, ethyl acetate extracts of fruit bodies, and mycelium of *H. erinaceus* have antimicrobial activity against a number of bacteria [16, 17, 19–22]. The antibacterial activity of the culture liquid of *H. erinaceus* was also found [22]. Besides, *H. erinaceus* extract showed strong activity against *Staphylococcus aureus* [16, 20, 23]. It should be noted that most data provide information about the antibacterial activity of extracts from the *H. erinaceus* fruiting bodies, while there is little information about the antimicrobial activity of extracts from mycelium and liquid fluid of other species of *Hericium* fungi [22]. Along with the antibacterial effect, the antifungal activity of extracts of mycelium of *Hericium* species against *Candida albicans* and *Cryptococcus neoformans* has also been reported [24].

In search of new sources of antimicrobial producers, the aim of our work was to study the antimicrobial activity of culture fluid and mycelial mass of three strains of three *Hericium* species from the IBK mushroom culture collection (Kyiv, Ukraine), selected after preliminary screening, as well as to evaluate the ability of *Hericium* fungi for biomass and extracellular polysaccharides production under submerged cultivation.

Materials and methods

Mushroom strains

Pure cultures of three species of *Hericium* fungi (*Hericium cirrhatum* 2393, *H. coralloides* 2332, *H. erinaceus* 2530) were obtained from the IBK Mushroom Culture Collection of the M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine (Table 1) [25]. All strains were maintained on agar wort medium and stored at 4 °C.

A liquid glucose-peptone-yeast nutrient medium (GPY) was used for fungal cultivation (g/L): glucose – 25.0; peptone – 3.0; yeast extract – 2.0; KH_2PO_4 – 1.0; K_2HPO_4 – 1.0; $MgSO_4$ $7H_2O$ – 0.25; pH 5.5.

The inoculum preparation was well described in our previous study [4]. Mycelium cultivation was carried out in 500 mL Erlenmeyer flasks with 100 mL of GPY liquid nutrient medium on an orbital shaker (120 rpm) at 25 ± 0.1 °C for 28 days.

Test microorganisms

Five bacterial strains and three fungal strains were tested in this research. Three Gram-negative bacteria include *Escherichia coli* (B-906), *Klebsiella pneumoniae* (M-123), and *Pseudomonas aureginosa* (B-900), two Gram-positive bacteria include *S. aureus* (B-918), *Bacillus subtilis* (B-901). All bacterial strains were obtained from the collection of the Faculty of Biotechnology and Microbiology of the National University of Food Technologies (Kyiv, Ukraine).

Fungal strains include *Aspergillus niger* (VURV-F 822), *Penicillium polonicum* (VURV-F 823) from the Collection of Microorganisms of Crop Research Institute (Prague, Czech Republic), and *Mucor globosus* (N-O18) from the collection of microorganisms of the Ukrainian Institute of Botany.

Mueller-Hinton Agar was used for growing bacteria (g/L): peptone, 17.0; starch - 15.0; yeast extract - 0.5; agar-agar, 17.0; pH 7. Bacteria were grown for 1 day at 37 ± 0.1 °C. Malt extract agar (MEA, Pronadisa, Spain)

Table 1: The studied species and strains of *Hericium.*

Species, IBK strain number	Origin and year of deposit
Hericium cirrhatum 2393	Isolated from a carpophore, Ukraine, Ivano-Frankivsk Region, Vorokhta, Carpathian National Nature Park, on *Fagus sylvatica*, 2015.
Hericium coralloides 2332	Isolated from a carpophore, Ukraine, Ivano-Frankivsk Region, Kosiv, Hutsulshchyna National Nature Park, Rocky Ridge, on *Fagus* sp. 2013.
Hericium erinaceus 2530	Obtained from Kharkiv Karazin National University, Kharkiv, Ukraine, 2017. Isolated from carpophore, Ukraine, Krim, Sebastopol region, Baydarskaya valley, vil. Peredovoe, on *Fagus taurica*, 2013.

was used for the micromycetes growth. Micromycetes were grown for 5 days at 26 ± 0.1 °C.

Preparation of mushroom extracts

Strains of *Hericium* were cultivated for 7, 14, 21, and 28 days in submerged conditions on an orbital shaker. After that, mycelial biomass was separated from the liquid by filtration, washed with distilled water, and dried to constant weight at 60 °C. Obtained fungal biomass was extracted with 70 % ethanol in a ratio of 1:50 (w/v, biomass to ethanol) in an ultrasonic bath at 40 °C for 30 min and left for 24 h at 4 °C. After that, the extract was filtered and centrifuged for 20 min at 3,500 rpm to remove the biomass. The resulting extract was tested for antimicrobial activity [26].

To concentrate the antimicrobial substances in the culture fluid, the method of liquid-liquid extraction was used. Ethyl acetate was added at a ratio of 2:1. The resulting mixture was shaken for 10 min and left for 20 h at 4 °C. The ethyl acetate fraction was evaporated to dryness on a rotary evaporator [27]. The procedure was repeated three times. The resulting residue was weighed, dissolved in 70 % ethanol, and adjusted to a volume equal to 1/10 of the original culture liquid.

Antimicrobial assay

The antibacterial activity of biomass and culture fluid extracts of the studied strains was assayed by the disk diffusion method [27]. A small number of bacteria from a freshly grown colony was transferred by a microbiological loop into test tubes with sterile saline, shaken until a homogeneous suspension was obtained, and the density of the inoculum was adjusted to exactly 0.5 in accordance with the McFarland standard (5×10^6 cells/mL) [26]. The resulting suspension of bacteria from the calculation of 0.2 mL was applied evenly to the surface of the Muller-Hinton medium. Extracts (10 µL) were applied to standard Bio Merieux firm discs (6 mm in diameter), dried at 40 °C for 30 min, and placed on the surface of an agar medium, pre-inoculated with a bacterial or fungal test culture. After that, the agar plates were incubated under appropriate conditions depending on the studied microorganism (24 h at 37 °C for bacteria and 72 h at 26 °C for fungi). Antibacterial/antifungal analysis was performed by measuring the growth inhibition zone around the filter discs [27]. In all assays, antibiotic Gentamycin sulphate (40 mg/mL, Ukraine) was used as a positive control. The ethyl acetate for the experiment with culture fluid and 70 % ethanol for the experiment with mycelial biomass were used as negative controls.

Analysis of the minimum inhibitory concentration (MIC) was carried out according to the broth microdilution method [28]. The test was performed in a 96-well polystyrene microtiter plate (Colstar, USA). 50 µL of the Muller-Hinton medium containing the appropriate concentration of the mushroom extract was added to each well, except for the control ones. The extracts were diluted in a range from 2 up to 768 times. After that, a 50 µL suspension of microbial cells (5×10^6 cells/mL) was added to each well; the sterility control well contained 100 mL of the Muller-Hinton medium, and the growth control well had 50 mL of medium and 50 mL of the bacterial suspension. The test was carried out in triplicate; MIC was defined as the minimum extract concentration at which no growth was observed in any repeats.

Polysaccharide determination

The mycelial biomass (MB) was harvested by filtration through Whatman No.1 filter paper. It was dried in a hot air oven at 60 °C until constant weights were recorded. For the determination of EPS, the obtained culture fluid after mycelia removal was concentrated in a vacuum evaporator three times from the initial volume, precipitated with 96 % cooled ethanol in a ratio of 1:1 and placed in a refrigerator at 4 °C for 24 h. The precipitate was isolated from the supernatant by centrifugation at 8,000 g for 15 min. After separation, EPS was dried at 60 °C to constant weight. The yields of MB and EPS were expressed as the g dry weight/L of the culture liquid [29, 30].

Statistical analysis

All experiments were carried out in triplicate. All data were statistically processed for analysis. The Student's *t*-test was applied to determine significance, and a p-value of <0.05 was considered statistically significant. The results were expressed as mean ± standard deviation using Excel 2010 (Microsoft Corporation, USA).

Results

Antibacterial and antifungal assay of *Hericium* fungi

The study of three *Hericium* species cultures (*H. cirrhatum* IBK 2393, *H. coralloides* IBK 2332, *H. erinaceus* IBK 2530) antibacterial activity and antifungal properties of culture fluid ethyl acetate extracts and mycelial mass aqueous-ethyl extracts against Gram-positive *B. subtilis* (B-901), *S. aureus* (B-918), and Gram-negative *E. coli* (B-906), *K. pneumoniae* (M-123), *P. aureginosa* (B-900) bacteria as well as *A. niger* (VURV-F 822), *P. polonicum* (VURV-F 823), and *M. globosus* (N-O18) fungi by the disk diffusion method was conducted. The mushroom strains were selected as a result of our preliminary antimicrobial screening of homogenized native mycelium and initial cultural liquid of 13 strains belonging to four *Hericium* species (*H. abietis*, *H. cirrhatum*, *H. coralloide*s, and *H. erinaceu*s) from the IBK Mushroom Culture Collection [31].

According to the results, none of the extracts showed any activity against test micromycete: *A. niger, P. polonicum,* and *M. globosus* under conducted cultivation conditions.

For all of the strains, we observed no antimicrobial activity of the homogenized mycelium and culture fluid after 7 days of submerged cultivation. It was also found that studied *Hericium* sp. strains did not show antimicrobial activity against test bacteria *Pseudomonas aeruginosa* and *E. coli*. An exception was only the culture fluid extracts from

H. coralloides IBK 2332 against *E. coli* on the 21st day of incubation.

Insignificant activity against Gram-negative bacteria *K. pneumoniae* was observed in strains *H. coralloides* IBK 2332 and *H. cirrhatum* IBK 2393 on the 14th - 21st days of incubation; the diameters of the growth retardation zone were less than 10 mm. The antimicrobial activity of fungal extracts against Gram-positive *B. subtilis* was also manifested on the 14th - 21st day of cultivation. The highest antimicrobial activity against *B. subtilis* was found in the culture fluid of the *H. erinaceus* IBK 2530 on the 14th day of cultivation; the diameter of the growth retardation zone was 28.0 ± 0.1 mm.

Among Gram-positive bacteria, *S. aureus* was the most sensitive to fungal extracts (Table 2, Figure 1). Ethyl acetate extracts of the culture fluid of *H. cirrhatum IBK* 2393 and *H. erinaceus IBK* 2530 showed the highest antimicrobial activity on the 14th day of cultivation, the diameter of the growth retardation zone was around 30 mm. The best results with the highest antimicrobial activity in growth suppression of *S. aureus* demonstrated the culture fluid extracts of *H. coralloides* IBK 2332 on the 21st day of cultivation; the diameter of the growth retardation zone was 32.1 ± 0.1 mm. Overall, these results indicate that in all investigated strains on the 14th and 21st day of cultivation, the growth retardation zone for *S. aureus* exceeded the positive control values. In contrast to the culture fluid, aqueous-ethyl extracts of the mycelial mass showed twice less antimicrobial activity, except *H. cirrhatum* IBK 2393 (Table 2).

Table 2: Antimicrobial activity of culture fluid ethyl acetate extracts and mycelial mass aqueous-ethyl extracts of *Hericium* strains against *Staphylococcus aureus.*

Duration of cultivation, days		14	21
Strain	**Extracts**	**Diameter of zone of inhibition, mm**	
Hericium cirrhatum	CF	29.9 ± 0.5	28.3 ± 0.2[c]
IBK 2393	MB	18.5 ± 0.2	21.0 ± 1.0[c]
Hericium coralloides	CF	30.2 ± 0.2	**32.1 ± 0.1**[c]
IBK 2332	MB	12.5 ± 0.4	15.5 ± 0.5[c]
Hericium erinaceus	CF	**30.2 ± 0.1**	28.0 ± 0.2[c]
IBK 2530	MB	14.2 ± 0.3	14.0 ± 0.2
Control +[a]			18.8 ± 0.1
Control -[b]			0

CF, ethyl acetate extracts of the culture fluid; MB, aqueous-ethyl extract of mycelial biomass. [a]Gentamycin sulphate, antibiotic solution; [b]ethyl acetate for experiment with culture fluid, 70 % ethanol for experiment with mycelial biomass. The maximum value is indicated in bold. All results are expressed as mean ± SD from three experiments (n=3). [c]The values within the columns between 14 and 21 days of cultivation are significantly different ($p \leq 0.05$).

The minimum inhibitory concentration of aqueous-ethyl extracts of *Hericium* fungi

For a better understanding of the prospects for the use of these extracts against bacterial pathogens, it is important to establish the minimum inhibitory concentrations of the extracts. The MIC is the lowest concentration of a sample that inhibits the visible growth of microorganisms. Since *Hericium* fungal extracts showed the maximal effect against *S. aureus*, therefore, MIC of this test culture was determined (Table 3). It was established that the extract of the *H. cirrhatum* biomass has the least inhibitory activity. So, the MIC for this extract was determined when it was diluted 6 times, which corresponds to the content of dry substances at the level of 1900 µg/mL. Extracts obtained from the biomass of *H. Coralloides* and *H. erinaceus* inhibited the growth of *S. aureus* at much higher dilutions, namely 96 times, the content of dry substances in these extracts was 130 µg/mL. The investigated extract of the culture liquid of *H. erinaceus* was more effective than the biomass extract of this mushroom; its MIC was 50 µg/mL.

Accumulation of biomass and extracellular polysaccharide production of *Hericium* fungi

Analyzing the data obtained, we note that all studied *Hericium* sp. strains synthesized extracellular polysaccharides in the glucose-peptone-yeast nutrient medium (Table 4).

In general, it is shown that for all strains, an increase in the accumulation of biomass was observed from 7 to 21 days of the cultivation period. Another important finding was that, at the same time, with an increase in the duration of cultivation, a decrease in the content of EPS was observed. The maximal value of biomass was produced by *H. cirrhatum* IBK 2393 on the 21st day of submerged cultivation - 17.45 ± 0.71 g/L. The maximum concentration of biomass produced by *H. coralloides* IBK 2332–11.53 ± 1.09 g/L and 12.10 ± 0.64 g/L was obtained on the 14th and 21st days of growth, respectively.

The data obtained indicate that after 7th day of submerged cultivation, all the strains studied produced the maximum EPS yield. The most promising EPS producers were strains of *H. coralloides* IBK-2332 (1.89 ± 0.12 g/L) and *H. erinaceus* IBK-2530 (1.90 ± 0.07 g/L).

Figure 1: Antibacterial activity of ethyl acetate extract of *Hericium* species culture fluid against *Staphylococcus aureus* (negative control, the 7th, 14th, 21st days of cultivation).

Table 3: Minimum inhibitory concentration (MIC) of extracts from biomass and liquid culture of *Hericium* fungi against *Staphylococcus aureus* (14 days of cultivation).

Strain	Type of extraction	Dilution	MIC[a], µg/mL
H. cirrhatum IBK 2393	biomass	6	1900
H. coralloides IBK 2332	biomass	96	130
H. erinaceus IBK 2530	biomass	96	130
	liquid culture	96	50

[a]Concentration was calculated in accordance with the content of dry matter in extracts.

Table 4: Accumulation of biomass and exopolysaccharides in *Hericium* fungi.

Strain	Time, days	Biomass, g/L	Exopolysaccharides, g/L
Hericium	7	4.16 ± 0.56	**1.55 ± 0.06**
cirrhatum	14	8.27 ± 0.29[a]	1.33 ± 0.02[a]
IBK 2393	21	**17.45 ± 0.71**[a]	0.53 ± 0.11[a]
	28	8.29 ± 0.9[a]	0.43 ± 0.05[a]
Hericium	7	5.59 ± 1.06	**1.89 ± 0.12**
coralloides	14	**11.53 ± 1.09**[a]	0.9 ± 0.13[a]
IBK 2332	21	**12.10 ± 0.64**[a]	0.55 ± 0.06[a]
	28	7.19 ± 0.31[a]	0.38 ± 0.05[a]
Hericium	7	2.31 ± 0.39	**1.90 ± 0.07**
erinaceus	14	6.23 ± 0.1[a]	**1.75 ± 0.1**
IBK 2530	21	**8.04 ± 0.42**[a]	0.39 ± 0.05[a]
	28	7.48 ± 0.11[a]	0.25 ± 0.03[a]

The maximum value is indicated in bold. All results are expressed as mean ± SD from three experiments (n=3). [a]The values within the rows in comparison with data obtained on the 7th day are significantly different ($p \leq 0.05$).

Discussion

In our studies, *H. coralloides* IBK 2332, *H. cirrhatum* IBK 2393, and *H. erinaceus* IBK 2530 extracts demonstrate strong antibacterial effects against bacterial test cultures. At the same time, analyzing the available literature, we did not find data indicating the antimicrobial activity of *H. cirrhatum* fungi. Previous studies demonstrate that *Hericium* fungi are producers of secondary metabolites with antimicrobial properties [2, 32–34]. An aqueous extract from the fruiting bodies of *H. erinaceus* is known to accelerate wound healing. Various researchers have confirmed the antimicrobial effect of extracts of fruiting bodies of *H. erinaceus* that inhibited the growth of *Bacillus cereus, B. subtilis, Enterococcus faecalis, S. aureus, P. aeruginosa, E. coli* and *Plesiomonas shigelloides* [6, 16, 23, 34–36]. Data obtained by Han et al. and Song et al. show that ethyl acetate and methanol extracts from *H. erinaceus* fruiting bodies exhibit a high antibacterial effect against *Micrococcus luteus* and *S. aureus*, and moderate activity against *Enterobacter cloacae* and *Streptococcus mutans.* Studies have shown that some of these metabolites have antibacterial and antifungal properties [21, 24]. It was found that higher antimicrobial activity of mushroom extracts was observed against Gram-positive bacteria, including drug-resistant strains, such as methicillin-resistant [23]. Okamoto et al. established the antimicrobial potential of *H. erinaceus* and identified some bioactive molecules with antimicrobial activity against *S. aureus, Salmonella enteritidis, Vibrio parahaemolyticus,* and *E. coli* [37]. Our results correspond to the literature. Analyzing the data obtained, we note that the studied strains

synthesize biologically active substances that are effective against the resistance to methicillin-resistant test bacterium *S. aureus*, which is known by various antibiotic resistance spectra.

Liu et al. studied various fractions of ethyl extracts of *H. erinaceus* mycelial biomass against *Helicobacter pylori*. All obtained samples had weak antibacterial activity (MIC from 250 to 500 μg/mL/mL) [38]. After purification and separation of the extracts by chromatography, an increase in the MIC to 12.5–50 μg/mL and 6.25–25 μg/mL was observed for fractions 1 and 2, respectively. Based on the obtained results, it can be assumed that during subsequent purifications of *H. erinaceus* IBK 2530 ethyl extract with MIC of 50 and 130 μg/mL to *S. aureus*, the antibacterial activity of the obtained samples increased. These data must be interpreted cautiously because the compositions of four fractions of *H. coralloides* ethyl acetate extract obtained in another research did not exhibit antibacterial activity against *B. subtilis, S. aureus, E. coli, Botrytis cinerea*, and *Rhizoctonia solani* [39].

Thus, comparing the obtained results with the literature data, it should be noted that *H. cirrhatum* IBK 2393, *H. coralloides* IBK 2332, and *H. erinaceus* IBK 2530 culture fluid extracts, as well as their biomass extracts, exhibit high antibacterial effect against Gram-positive bacteria such as *S. aureus*, as well as *B. subtilis,* which makes it possible to talk about the prospects of using these mushroom strains in the future as antibacterial agents. However, this requires further study and is another potential area of future research.

So far, many studies have focused on extracts obtained from fruiting bodies [1, 2, 6, 8, 16–18, 32, 34–36]. The current research was conducted by submerged cultivation, and fungal mycelium and culture fluid were used for extract preparation.

According to the literature data, the antibacterial effect of basidiomycetes is due to the presence of different biologically active compounds, including polysaccharides [1, 4, 7]. Previous studies have reported that the yield of extracellular polysaccharides during the cultivation of *H. erinaceus* ranges from 0.33 to 2.77 g/L depending on the carbon and nitrogen sources, minerals, vitamins, and the pH of the nutrient medium. Under optimal cultivation conditions, the maximum biomass yield in *H. erinaceus* reached 14.24 ± 0.45 g/L. At the same time, the maximum EPS production was observed on the 8th day of fungal cultivation (2.75 ± 0.27 g/L) [13]. In our research, the maximum biomass yield in *H. erinaceus* IBK 2530 was 8.04 ± 0.42 g/L, and the maximum EPS production we also observed after 7 days of fungal cultivation was 1.90 ± 0.07 g/L. Compared with literature data on other mushrooms, the maximum EPS production in *Lentinus edodes* was 0.8 g/L, in *Pleurotus ostreatus* 1.3 g/L, and in *P. eryngii* 1.0 g/L [29]. The results strongly depend on the effect of various sources of carbon and nitrogen on the productivity of biomass and extracellular polysaccharides, as was shown by Elisashvili et al. [29]. The highest EPS productivity was obtained on media with glucose or sodium gluconate as the carbon source and peptone or liquid corn extract as the nitrogen source [29]. Our study did not aim to optimize the medium and increase biomass and EPS yield. Therefore, we used the composition of the media components whose effectiveness was proven by previous researchers: glucose 25.0 g/L, peptone 3.0 g/L, and yeast extract 2.0 g/L [29, 30]. It was a screening study of promising *Hericium* species and strains selected at the previous stage of our research. The most promising results were 17.45 ± 0.71 g/L and 12.10 ± 0.64 g/L biomass produced by *H. cirrhatum* IBK 2393 and *H. coralloides* IBK 2332, respectively, on the 21st day of submerged cultivation. According to our data, the EPS accumulation in culture liquid rather correlated with mushroom biomass yield. Interestingly, long-term submerged cultivation of these cultures to obtain EPS is not advisable. Among mushrooms tested, the maximum EPS we observed on the 7th of submerged cultivation. Similar results have been reported by Elisashvili after 7 days of cultivation of *Agaricus* sp. and *Oudemansiella canarii* [3].

In conclusion, *Hericium* extracts exhibit strong antibacterial activity against the Gram-positive bacteria *S. aureus*. Our results demonstrate that mushroom extracts had no effect against *A. niger*, *P. polonicum*, and *M. globosus*. On the 7th day of submerged cultivation, all the strains studied produced the maximum EPS yield (1.55–1.9 g/L). However, the EPS productivity of each strain decreased with the length of cultivation time. At the same time, an inverse correlation was observed for the accumulation of mycelial biomass. The maximum amount of biomass was accumulated on 14–21 days of fungal cultivation. Thus, *Hericium* fungi have significant potential for the development of new drugs with health benefits properties.

Research funding: This research was funded by the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan (Grant No. AP09258296).

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: The local Institutional Review Board deemed the study exempt from review.

References

1. Wasser SP. Medicinal mushroom science: current perspectives, advances, evidences, and challenges. Biomed J 2014;37:345–56.
2. Agrawal DC, Dhanasekaran M, editors. Medicinal mushroom. Recent progress in research, 1st ed. Singapore: Springer; 2019.
3. Elisashvili V. Submerged cultivation of medicinal mushrooms: bioprocesses and products. Int J Med Mushrooms 2012;14:211–39.
4. Wasser SP, editor. Biological features of medicinal macromycetes in culture. Kiev: Alterpres; 2012.
5. Friedman M. Chemistry, nutrition, and health-promoting properties of *Hericium erinaceus* (lion's mane) mushroom fruiting bodies and mycelia and their bioactive compounds. J Agric Food Chem 2015;63:7108–23.
6. Thongbai B, Rapior S, Hyde KD, Wittstein K, Stadler M. Hericium erinaceus, an amazing medicinal mushroom. Mycol Prog 2015;14:1–23.
7. Dmytriev OP. Botany and mycology: modern horizons. Kyiv: Nash format; 2021:564 p.
8. Mizuno T. Bioactive substances in Hericium erinaceus. Pers. (Yamabushitake), and its medicinal utilization. Int J Med Mushrooms 1999;1:105–19.
9. Reshetnikov SV, Wasser SP, Tan KK. Higher Basidiomycota assource of antitumor and immunostimulating polysaccharides. Int J Med Mushrooms 2001;3:361–94.
10. Choudhary S. Characterization and applications of mushroom exopolysaccharides. In: Singh J, Gehlot P, editors. New and future developments in microbial biotechnology and bioengineering. Recent advances in application of fungi and fungal metabolites: environmental and industrial aspects. Amsterdam: Elsevier B.V.; 2020.
11. Wang JC, Hu SH, Su CH, Lee TM. Antitumor and immunoenhancing activities of polysaccharide from culture broth of Hericium spp. Kaohsiung J Med Sci 2001;17:461–7.
12. Wang XY, Zhang DD, Yin JY, Nie SP, Xie MY. Recent developments in Hericium erinaceus polysaccharides: extraction, purification, structural characteristics, and biological activities. Crit Rev Food Sci Nutr 2019;59: 96–115.
13. Malinowska E, Krzyczkowski W, Lapienis G, Herold F. Improved simultaneous production of mycelial biomass and polysaccharides by submerged culture of Hericium erinaceum: optimization using a central composite rotatable design (CCRD). J Ind Microbiol Biotechnol 2009;36:1513–27.
14. Wu DT, Li WZ, Chen J, Zhong QX, Ju YJ, Zhao J, et al. An evaluation system for characterization of polysaccharides from the fruiting body of Hericium erinaceus and identification of its commercial product. Carbohydr Polym 2015;124:201–7.
15. Wittstein K, Rascher M, Rupcic Z, Lowen E, Winter B, Koster RW, et al. Corallocins A-C, nerve growth, and brain-derived neurotrophic factor-inducing metabolites from the mushroom Hericium coralloides. J Nat Prod 2016;79:2264–9.
16. Wong KH, Sabaratnam V, Abdullah N, Kuppusamy UR, Naidu M. Effects of cultivation techniques and processing on antimicrobial and antioxidant activities of Hericium erinaceus (Bull.: Fr.) Pers. extracts. Food Technol Biotechnol 2009;47:47–55.
17. Shang X, Tan Q, Liu R, Yu K, Li P, Zhao GP. In vitro anti-Helicobacter pylori effects of medicinal mushroom extracts, with special emphasis on the Lion's Mane mushroom, Hericium erinaceus (Higher Basidiomycetes). Int J Med Mushrooms 2013;15:165–74.
18. Shen H-S, Shao S, Chen J-C, Zhou T. Antimicrobials from mushrooms for assuring food safety. Compr Rev Food Sci Food Saf 2017;16:316–29.
19. Mustafin KG, Bisko NA, Blieva RK, Al-Maali GA, Krupodorova T, ZhB N, et al. Antioxidant and antimicrobial potential of Ganoderma lucidum and Trametes versicolor. Turk Biyokim Derg 2022;47: 483–9.
20. Kim DM, Pyun CW, Ko HG, Park WM. Isolation of antimicrobial substances from Hericium erinaceum. Mycobiology 2000;28:33–8.
21. Han SR, Jun JA, Yang HS, Oh TJ. Comparison of physiological activity of solvent extracts from Hericium erinaceus. Indian J Sci Technol 2015;8: 1–7.
22. Krupodorova TA, Barshteyn VY, Zabeida EF, Pokas EV. Antibacterial activity of macromycetes mycelia and culture liquid. Microbiol Biotechnol Lett 2016;44:246–53.
23. Nikolovska-Nedelkoska D, Atanasova-Pancevska N, Karadelev MP, Kungulovski DV. Bactericidal activities of selected macrofungi extracts against Staphylococcus aureus. Zb Matice Srp Prir Nauk 2017;133: 193–200.
24. Song X, Gaascht F, Schmidt-Dannert C, Salomon CE. Discovery of antifungal and biofilm preventative compounds from mycelial cultures of a unique North American Hericium sp. fungus. Molecules 2020;25:963.
25. Bisko N, Lomberg M, Mykchaylova O, Mytropolska N [Online]. IBK mushroom culture collection. In: Version 1.5. The IBK mushroom culture collection of the M.G. Kholodny Institute of Botany; 2022. Available from: https://www.gbif.org/dataset/148aefb6-cf88-443e-9780-b774ef7333e7 [Accessed via GBIF.org on 13 Jan 2023].
26. Mykchaylova O, Poyedinok N. Antimicrobial activity of Fomitopsis officinalis. Bondartsev & singer in pure culture. Innov Biosyst Bioeng 2021;5:220–7.
27. Dyakov MY, Kamzolkina OV, Shtaer OV, Bis'ko NA, Poedinok NL, Mikhailova OB, et al. Morphological characteristics of natural strains of certain species of basidiomycetes and biological analysis of antimicrobial activity under submerged cultural conditions. Microbiology 2011;80:274–85.
28. Wiegand I, Hilpert K, Hancock RE. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc 2008;3:163–75.
29. Elisashvili V, Wasser SP, Tan K-K, Chichua D, Kachlishvili E. Extracellular polysaccharide production by culinary-medicinal shiitake mushroom Lentinus edodes species depending on carbon and nitrogen source. Int J Med Mushrooms 2004;6:165–72.
30. Babitskaya VG, Scherba VV, Mytropolskaya NY, Bisko NA. Exopolysaccharides of some medicinal mushrooms: production and composition. Int J Med Mushrooms 2000;2:51–4.
31. Lomberg M, Krupodorova T, Krasinko V, Mykchaylova O. Antibacterial activity of culture filtrates and mycelia of the selected strains of macromycetes from genus Hericium. Bot Serb 2023;2:1–21.
32. Alves MJ, Ferreira ICFR, Dias J, Teixeira V, Martins A, Pintado M. A review on anti-microbial activity of mushroom (Basidiomycetes) extracts and isolated compounds. Planta Med 2012;78:1707–18.
33. De Silva DD, Rapior S, Sudarman E, Stadler M, Xu J, Alias SA, et al. Bioactive metabolites from macrofungi: ethnopharmacology, biological activities, and chemistry. Fungal Divers 2013;62:1–40.
34. Hyde KD, Xu J, Rapior S, Rapior S, Lumyong S, Niego AGT, et al. The amazing potential of fungi: 50 ways we can exploit fungi industrially. Fungal Divers 2019;97:1–136.
35. Khan MA, Tania M, Liu R, Rahman MM. Hericium erinaceus: an edible mushroom with medicinal values. J Integr Complement Med 2013;10: 253–8.
36. Jiang S, Wang S, Sun Y, Zhang Q. Medicinal properties of Hericium erinaceus and its potential to formulate novel

mushroom-based pharmaceuticals. Appl Microbiol Biotechnol 2014; 98:7661–70.
37. Okamoto K, Sakai T, Shimada A, Shirai R, Sakamoto H, Yoshida S, et al. Antimicrobial chlorinated orcinol derivatives from mycelia of Hericium erinaceum. Phytochemistry 1993;34:1445–6.
38. Liu JH, Li L, Shang XD, Zhang JL, Tan Q. Anti-Helicobacter pylori activity of bioactive components isolated from Hericium erinaceus. J Ethnopharmacol 2016;183:54–8.
39. Kim JY, Woo E-E, Lee IK, Yun BS. New antioxidants from the culture broth of Hericium coralloides. J Antibiot 2018;71:822–5.