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The antibacterial activity of culture filtrates and mycelia of selected strains of macromycetes from the genus *Hericium*

Margarita Lomberg[1*], Tetiana Krupodorova[2], Viktoriia Krasinko[3] and Oksana Mykchaylova[1]

1 M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine, Tereshchenkivska Str., 2, 01601, Kyiv, Ukraine
2 Institute of Food Biotechnology and Genomics of the National Academy of Sciences of Ukraine, Baidy-Vyshnevetskogo Str. 2a, 04123, Kyiv, Ukraine
3 National University of Food Technologies, Volodymyrska Str., 68, 01601, Kyiv, Ukraine
* Corresponding author: margarita@lomberg.kiev.ua

ABSTRACT:
The aim of the study was to investigate the antibacterial activity of selected strains of the genus *Hericium,* belonging to basidiomycetes, from the IBK Mushroom Culture Collection of the M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine. A total of 14 strains including *H. abietis*, *H. cirrhatum*, *H. coralloides*, and *H. erinaceus* were investigated. The strains were cultivated on a liquid glucose-peptone-yeast medium. Both the homogenised mycelium and filtrate of these fungi were evaluated against gram-positive (*Bacillus subtilis*, *Micrococcus luteus*, and *Staphylococcus aureus*) and gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria by the disk diffusion and cylinder methods. The activity of the strains varied significantly. Depending on the techniques assay, species, strain, and stage of fungal development, the inhibition zone of the tested bacteria ranged from 5.3 mm to 20.0 mm. In general, the antibacterial potential of the culture filtrates of the investigated species was significantly higher than their mycelia activity. The homogenised mycelium showed potentially good results only against *E. coli*. The antibacterial activities of the *H. abietis* and *H. cirrhatum* species were observed for the first time. To the best of our knowledge, the ability of *H. coralloides* to inhibit the growth of *P. aeruginosa* and *S. aureus* has not been previously reported. The obtained results indicate the ability of the studied *Hericium* species to produce antibacterial metabolites with a wide and narrow spectrum of action which might have potential health benefits and could be recommended for the further analysis, isolation and identification of potentially promising antibacterial compounds in pharmacology.

Keywords:
antibacterial action, basidiomycetes, culture filtrate, mycelium.

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INTRODUCTION

The emergence of multidrug-resistant forms of pathogens (Prestinaci *et al.* 2015; Terreni *et al.* 2021), as well as the frequent occurrence of side effects from drugs which are commercially approved, creates the demand for the further search of new antimicrobial compounds, especially antibiotics of natural origin (Rani *et al.* 2021). Nowadays, there is a constant interest in the study of fungi and the possibility of obtaining different biologically active substances from fruiting bodies, mycelia, and culture filtrates. The medicinal properties of certain species of fungi have been known for a long time, including anticancer, antioxidant, immunomodulatory,

radioprotective and anti-stress activities. In practice, antibiotic substances isolated from fruiting bodies, mycelium biomass, and culture filtrates are used (Hyde *et al.* 2019).

The urgent need for effective and safe pharmaceuticals promotes the constant search and monitoring of new species and strains of fungi from different ecological and trophic groups. Due to this fact, preference is given to the edible species of fungi, e.g., all known species of the *Hericium* genus. *Hericium erinaceus* (Bull.: Fr.) Pers. is one of the most well-known species of the *Hericium* genus, which belongs to *Hericiaceae*, *Russulales*, *Agaricomycetes*, *Agaricomycotina*, *Basidiomycota*, *Dikarya*, *Fungi* (MycoBank 2023). The effectiveness of this species has been confirmed in clinical practice and has long been used in the traditional medicine of Southeast Asia (Kawagishi & Zhuang 2008). There is extensive information on the health benefits of *H. erinaceus* and its bioactive compounds have been summarised in several studies (Kawagishi & Zhuang 2008; Khan *et al.* 2013; Thongbai *et al.* 2015; Wang *et al.* 2015; Sokół *et al.* 2016). Numerous studies have reported various medicinal properties of this mushroom such as anticancer, antihypertensive, anti-inflammatory, antimicrobial, antioxidant, cytotoxic, gastroprotective, hepatoprotective, hypolipidemic, immunomodulating, neuroprotective, neuroregenerative, and wound-healing activities (Kawagishi & Zhuang 2008; Khan *et al.* 2013; Han 2015; Thongbai *et al.* 2015; Wang *et al.* 2015; Sokół *et al.* 2016; Wong *et al.* 2016; Spelman 2017; Zhang *et al.* 2017; Rupcic *et al.* 2018; Li *et al.* 2019; Song *et al.* 2020; Lomberg 2021).

Another well-known species of this genus is *Hericium coralloides* (Scop.) Pers. The medicinal properties of this fungus compare favourably with *H. erinaceus* due to the presence of erinacine in its mycelium. Erinacine exhibits different therapeutic effects including analgesic and antimicrobial properties, and has also been found to aid in the restoration of the nerve growth factor (Saito *et al.* 1998; Mizuno 1999; Mori *et al.* 2010; Thongbai *et al.* 2015; Wittstein *et al.* 2016).

Although the antimicrobial properties of *Hericium* species have been studied by various scientists, these studies have been rather unsystematic, and as a result, there is still insufficient information about the full potential of the genus *Hericium*. Also, it should be mentioned that most research contains data on the antibacterial activity of extracts (aqueous, methanol, ethanol, ethyl acetate) obtained from *H. erinaceus* fruiting bodies (Okamoto *et al.* 1993; Kawagishi 2005; Wong *et al.* 2009; Shang *et al.* 2013; Han *et al.* 2015; Nikolovska-Nedelkoska *et al.* 2017). There have been fewer studies on the antibacterial properties of *H. coralloides*. The presence of antimicrobial activity was detected in the aqueous extracts of *H. coralloides* fruiting bodies against *E. coli*, *M. luteus* and *B. subtilis,* while only the ethanol extract inhibited the growth of *B. subtilis* (Pasaylyuk 2017). However, as far as we know, there is no data on the antibacterial activity of other *Hericium* species. The aim of this research is to evaluate the antibacterial activity of culture filtrates and mycelia of the selected strains of *Hericium abietis, H. cirrhatum, H. coralloides, and H. erinaceus.*

MATERIALS AND METHODS

Investigated fungal strains. Pure cultures of four species of the *Hericium* genus (14 strains) of different geographical origin (Table 1) were obtained from the Mushroom Culture Collection of the M.G. Kholodny Institute of Botany (IBK) of the National Academy of Sciences of Ukraine (Bisko *et al.* 2022). Some of the investigated strains are deposited in the NCBI database available at GenBank (https://www.ncbi.nlm.nih.gov/genbank/) as follows: MN646242 - isolate *Hericium abietis* IBK 2376; MG549950 - isolate *Hericium coralloides* IBK 1876, MG549951 - IBK 2332 and MG549952 - IBK 2333; MN646239 and MN646241 – isolates *Hericium erinaceus* IBK 977 and IBK 2239 respectively. The stock cultures were maintained on beer wort agar (WA - liquid beer wort, diluted to a density of 8° Balling scale with distilled water, and 20 g/L agar) slants at 4**°C**.

Cultivation conditions. The components of the glucose-peptone-yeast (GPY) culture medium were as follows: 25 g/L glucose, 3 g/L peptone, 2 g/L yeast extract, 1 g/L K_2HPO_4, 1 g/L KH_2PO_4, and 0.25 g/L $MgSO_4 \times 7H_2O$ in distilled water. The culture medium was adjusted to pH 6.0. A volume of 50 mL of GPY liquid medium in 250-mL Erlenmeyer flasks was inoculated with 3 mycelium discs (diameter 8 mm) from 10-day-old pure cultures of the investigated *Hericium* strains grown in Petri dishes on GPY agar medium and then incubated for 7 or 14 days at 26 ± 2°C under static conditions.

Separation. The mycelium was separated from the medium by filtering through Whatman filter paper No. 4. Then the mycelial mass was washed with distilled water. A total of 5 g of mycelial mass was ground and homogenised using a mortar and pestle. The initial culture filtrate was obtained after the separation of the biomass. Culture filtrates of six selected strains of different *Hericium* species: *H. abietis* IBK 2376, *H. coralloides* IBK 1876, 2333, and *H. erinaceus* 339, 965, 2239 with very low antibacterial activity were concentrated. This was then evaporated in a sand bath until the volume of the solution was reduced by a factor of 10. The mycelium and culture filtrates were collected after 7 and 14 days for the antimicrobial experiments.

Bacterial species and culture condition. Gram-positive bacteria *Bacillus subtilis*, *Staphylococcus aureus*, and *Micrococcus luteus*, and gram-negative bacteria *Escherichia coli,* and *Pseudomonas aeruginosa* were collected

Table 1. The *Hericium* species and strains of different geographical origin used in the study

Species	Strain	Country of origin	Year of deposit
Hericium abietis (Weir ex Hubert) K.A. Harrison	2376	Ukraine	2014
Hericium cirrhatum (Pers.) Nikol.	2393	Ukraine	2015
Hericium coralloides (Scop.) Pers.	1876	Ukraine	2008
	2332	Ukraine	2013
	2333	Ukraine	2013
Hericium erinaceus (Bull.) Pers.	339	Ukraine	1988
	963	Japan	1996
	965	Germany	1996
	977	Czech Republic	1981
	991	Belgium	1997
	992	Germany	1997
	2239	USA	2013
	2530	Ukraine	2016
	2536	Vietnam	2016

from the Microbial Culture Collection of the National University of Food Technologies. The tested microorganisms were cultured on meat-peptone agar MPA: (5 g/L peptone, 1.5 g/L meat extract, 1.5 g/L yeast extract, and 20 g/L agar) (37°C, 24 h). Each microorganism was suspended in a sterile saline solution and diluted to 10^4 colony forming units (CFU) per mL.

Antibacterial assay. The antibacterial activity was determined using the agar paper disc diffusion and cylinder methods (Bilay 1982; Dyakov *et al.* 2011). Sterile paper discs (8 mm in diameter) or three stainless steel cylinders of uniform size (6 × 10 mm) were used. The suspension of homogenised native mycelium (50 µl), initial cultural filtrate (50 µL) or 10-fold concentrated culture liquid (50 µL) of the tested fungal strains were applied to the disks, dried at 40°C for 30 minutes and placed into Petri dishes with MPA previously inoculated with bacterial suspensions. For the cylinder method, 100 µL of homogenized mycelium or 100 µL of cultural filtrate were used, respectively. The inoculated Petri dishes were incubated overnight at 37°C. In all the assays, the fungi-free GPY culture media was used as the negative control, while the broad-spectrum antibiotic Gentamycin sulphate (40 mg/mL, Ukraine) was used as the positive control. Full inhibition of the growth of all the investigated test bacteria was observed.

The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the disc or cylinder (in mm) including their diameters. The readings were taken in three different fixed directions in all 3 replicates and the average values were tabulated. To simplify the interpretation of the results, the following scales were used: 0 (inactive), no inhibition or zone inhibition values of less than 8 mm or 6 mm in the case of the disc diffusion or cylinder methods, respectively; 1 (weakly active), zone inhibition between 8 or 6 and 12 mm; 2 (active), zone inhibition between 13 and 18 mm; 3 (strongly active) zone inhibition greater than 18mm.

Statistical analysis. The experimental results were expressed as means ± SEM (standard error of the mean) of triplicates. Statistical analysis was performed using the Fisher's LSD test. Differences at $P < 0.05$ were considered to be significant. The data was analysed in Excel using Microsoft Office XP software, Statistical Package for Social Sciences, version 11.5 (SPSS Inc., Chicago, 2002).

RESULTS

The metabolites produced by four *Hericium* species (a total of 14 strains) exhibiting antibacterial properties were studied by the disk diffusion method. The presence of antibacterial activity was observed in all the species included in the study. However, the activity of the initial culture filtrates from most of the investigated cultures against the test bacteria was absent. The activity of the five selected strains against *E. coli* and *S. aureus* was established only after 10-fold concentration of their culture filtrate. The antibacterial properties of *H. abietis* and *H. cirrhatum* species were observed for the first time. The data relating to the antibacterial properties of the investigated fungi after 14 days of cultivation are summarised in Table 2.

No antimicrobial activity was observed after seven days of cultivation of the homogenised mycelium and culture filtrate of all the strains. Consequently, only the data received after 14 days of cultivation is presented in Table 2.

The activity of the strains varied and the zones of inhibition against the pathogens ranged from 8.2 mm to 14.8 mm. The highest inhibitory activity was found against *B. subtilis* in the culture filtrate of *H. cirrhatum* (strain 2393) – 13.0 ± 1.2 mm, against *E. coli* – in the culture filtrate of *H. coralloides* (strain 2333) after its concentration – 14.0 ± 1.0 mm, against *M. luteus* - in the culture filtrate of *H. erinaceus* (strain 2530) – 12.4 ± 0.4 mm and against *S. aureus* - in the culture filtrate of *H. erinaceus* (strain 2239) after its concentration – 13.2 ± 1.6 mm. The maximum growth suppression of *P. aeruginosa* was demonstrated by the homogenised mycelium of *H. erinaceus* (strains 977 and 992) – 11.4 ± 0.1 mm and 11.4 ± 0.8 mm respectively. Overall, the antibacterial potential of the culture filtrates of the investigated species was significantly higher than their mycelial activity. In some cases, the homogenized mycelium and culture filtrates of the fungi were able to inhibit bacterial growth at the

Table 2. The antibacterial activity of the *Hericium* species by the disk diffusion method.

Fungal species	Fungal strain		The zone of inhibition (mm) obtained using the fungal cultures which have been cultivated for 14 days				
			Bacillus subtilis	*Escherichia coli*	*Micrococcus luteus*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Hericium abietis	2376	HM	0	10.6±0.6[a]	–	–	0
		CF	0	0	–	–	12.0±0.0[a,*]
Hericium cirrhatum	2393	HM	9.6±0.4[a]	12.0±0.6[b]	10.0±0.2[a]	–	–
		CF	13.0±1.2[b]	–	–	10.0±0.5[a]	–
Hericium coralloides	1876	HM	0	10.0±0.0[a]	–	–	0
		CF	0	0	–	–	10.0±0.0[a,*]
	2332	HM	8.8±0.4[a]	10.0±0.5[b]	12.0±0.9[c]	9.0±0.1[a]	–
		CF	11.4±0.6[b]	9.6±0.2[a]	12.0±1.0[b]	9.2±0.8[a]	–
	2333	HM	0	0	–	–	0
		CF	0	14.0±1.0[a,*]	–	–	0
Hericium erinaceus	339	HM	0	0	–	–	0
		CF	0	11.2±0.5[a,*]	–	–	11.6±0.8[a,*]
	963	HM	10.4±0.2[c]	11.6±0.8[d]	9.0±0.0[a]	9.6±0.4[b]	–
		CF	0	9.0±0.2[a]	–	10.0±0.5[b]	–
	965	HM	0	10.4±0.5[a]	–	–	0
		CF	0	0	–	–	0
	977	HM	10.6±0.4[a]	0	–	11.4±0.1[b]	–
		CF	12.4±1.0[b]	9.0±0.0[a]	11.0±0.5[b]	9.0±0.2[a]	–
	991	HM	11.6±0.5[c]	10.0±0.0[b]	10.6±0.4[b]	9.4±0.2[a]	–
		CF	10.6±0.4[b]	9.0±0.1[a]	12.0±0.6[c]	9.0±0.3[a]	–
	992	HM	11.0±0.5[b]	10.0±0.3[a]	9.6±0.4[a]	11.4±0.8[b]	–
		CF	10.0±0.3[b]	9.0±0.2[a]	10.0±0.5[b]	9.4±0.0[a]	–
	2239	HM	0	0	–	–	0
		CF	0	11.0±0.0[a,*]	–	–	13.2±1.6[b,*]
	2530	HM	11.0±0.1[b]	–	10.0±0.4[a]	–	–
		CF	10.0±0.3[b]	0	12.4±0.4[c]	8.4±0.0[a]	–
	2536	HM	8.2±0.0[a]	10.6±0.8[c]	–	8.8±0.5[b]	–
		CF	12.0±0.6[c]	10.8±0.2[b]	11.0±0.0[b]	9.0±0.4[a]	–

Notes: HM- homogenised mycelium; CF- culture filtrate; "–" no data available; «*» the activity of the strains was found only after culture filtrate concentration; the values accompanied by the same letters are not significantly different ($P \leq 0.05$) according to Fisher's LSD test.

same level. This tendency was shown by *H. coralloides*, strain 2332 against *E. coli*, *M. luteus*, *P. aeruginosa*, and *H. erinaceus*, strain 2536 – against *E. coli* and *P. aeruginosa*, and strain 992 – against *M. luteus*. In general, the antibacterial activity of the *H. erinaceus* strains was slightly higher compared to other *Hericium* species and strains in the primary screening. This allowed us to select the *H. erinaceus* strains for further studies.

The activity of the *Hericium erinaceus* strains against the tested pathogens varied greatly and showed zones of inhibition ranging from 5.3 mm to 20.0 mm by the cylinder method (Table 3). In addition, we also observed the dependence of the intensity of the antibacterial properties on the duration of fungi cultivation.

A clear pattern has not been established. However, a noticeable tendency can be observed as when the duration of fungi cultivation time increases, the antibacterial activity against the test cultures of *E. coli* and *M. luteus* mainly increases, while the antibacterial activity against *P. aeruginosa* decreases. Nevertheless, the best results were obtained on the 7th day of cultivation: the homogenised mycelium of *H. erinaceus*, strain 2536, exhibited the highest inhibitory activity against *E. coli*, while the culture filtrate of strain 2530 was more effective in suppressing *P. aeruginosa* growth. Furthermore, the homogenised mycelium and culture filtrate of strain2530, obtained on the 7th day of cultivation, strongly inhibited the growth of *M. luteus*. These results were close to the maximum suppression of *M. luteus* growth by the culture filtrate of strain 2350 and the homogenised mycelium of strain 977 which were investigated on the 14th day of cultivation. *M. luteus* was the most sensitive to

Table 3. Antibacterial activity of *Hericium erinaceus* strains on 7 and 14 days of cultivation by the cylinder method

Fungal strains		Zone of inhibition (mm) obtained using fungal cultures					
		Escherichia coli		*Pseudomonas aeruginos*a		*Micrococcus luteus*	
		Term of cultivation the fungal cultures, days					
		7	14	7	14	7	14
Hericium erinaceus 977	HM	8.0±0.1^{b}	17.5±0.4^{d}	9.5±0.2^{c}	4.8±0.2^{a}	14.3±1.0^{c}	19.2±0.5^{e}
	CF	12.3±0.2^{d}	13.0±0.2^{e}	10.7±0.7^{c}	7.0±0.5^{a}	7.7±0.6^{a}	8.3±0.2^{b}
Hericium erinaceus 2530	HM	7.8±0.0^{b}	14.3±0.3^{d}	7.3±0.2^{a}	12.8±0.2^{c}	20.0±1.1^{e}	7.5±0.4^{a,b}
	CF	16.0±0.4^{d}	7.8±0.2^{a}	14.0±0.0^{c}	8.7±0.4^{b}	18.5±0.5^{e}	21.2±1.2^{f}
Hericium erinaceus 2536	HM	19.0±0.5^{f}	16.7±0.3^{d}	13.0±0.3^{b}	5.3±0.4^{a}	15.0±0.2^{c}	17.8±0.6^{e}
	CF	8.7±0.2^{b}	12.8±0.1^{d}	11.0±0.5^{c}	7.3±0.6^{a}	14.3±0.1^{e}	14.0±0.5^{e}

Notes: HM- homogenised mycelium, CF- culture filtrate; the values accompanied by the same letters are not significantly different ($P \leq 0.05$) according to Fisher's LSD test.

the activity of the tested strains of *H. erinaceus*, while *P. aeruginosa* was the least sensitive.

Generally, the obtained results from the disc-diffusion method indicate the weak antibacterial activity of the investigated *Hericium* species, with the exception of the culture filtrates of *H. cirrhatum* (strain 2393), *H. coralloides* (strain 2333), and *H. erinaceus* (strain 2239) against *B. subtilis*, *E. coli* and *S. aureus* respectively (Table 2). Th disk diffusion method is the most flexible susceptibility testing method in terms of the search for antimicrobial agents. The usage of the cylinder method as an assay technique allowed us to increase the activity of the *H. erinaceus* strains from weakly active to active and strongly active (Tables 2 & 3). This tendency was found in the case of the homogenised mycelium as well as culture filtrates of *H. erinaceus* (strains 977, 2530, 2536) obtained on the 14th day of growth against *E. coli*. A similar inclination was observed for *H. erinaceus*, strain 2530, and the homogenised mycelium of strain 977 against *P. aeruginosa*. This trend was also observed in the case of *H. erinaceus*, strain 2566, the culture filtrate of strain 2530 and the homogenised mycelium of strain 977 against *M. luteus*. These improved results can probably be explained by the fact that cylinders hold a greater volume of fungi samples than disks.

DISCUSSION

Basidiomycetes as a source of natural antibiotics attract increasingly greater attention among researchers. The metabolites extracted from some *Hericium* species exhibited antibacterial activity. It is known that the mycelium as well as the culture filtrate of *H. clathroides* and *H. flagellum* inhibited the growth of *Bacillus subtilis* and *Candida pseudotropicalis* (Semerdžieva & Veselsky 1986). Ethanol extracts of *H. erinaceus* mycelia exhibited antimicrobial activity against *B. subtilis*, *Verticillium dahlia*, *Aspergillus niger*, and *Saccharomyces cerevisiae* (Okamoto *et al.* 1993). The mycelium extract of *H. erinaceus* was effective against *Staphyloccocus aureus* (Kim *et al.* 2000). Ethanol extracts of the fruiting bodies and the mycelia of *H. erinaceus* also exhibited activity against *S. aureus* (MRSA) (Kawagishi 2005). Methanol extracts of *H. erinaceus* fruiting bodies showed antimicrobial activity against *Bacillus cereus*, *B. subtilis*, *S. aureus*, *Enterococcus faecalis, Salmonella* sp., *S. enterica serovar Typhimurium*, *Shigella* sp., *S. flexneri*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Plesiomonas shigelloides*, *Candida albicans*, *C. parapsilosis*, and *Schizosaccharomyces pombe* at different levels from low to high (Wong *et al.* 2009). Ethanol and ethyl acetate extracts of *H. erinaceus* fruiting bodies displayed inhibitory action against *Helicobacter pylori* associated with chronic gastritis and gastric ulcers in patients (Shang *et al.* 2013). Ethyl acetate extracts from *H. erinaceus* fruiting bodies showed fairly high activity against *Micrococcus luteus*, while methanol extracts exhibited strong activity against *S. aureus* and *M. luteus*, and moderate action against *Enterobacter cloacae*. In addition, weak antibacterial effects were also demonstrated against *Streptococcus mutans* and *S. sanguinis*, bacteria associated with dental caries (Han *et al.* 2015). The antibacterial activity of *H. erinaceus* culture filtrates was observed against *B. subtilis* and *S. aureus*, as well as in *H. erinaceus* mycelium against *E. coli* (Krupodorova *et al.* 2016). The examined ethanol extracts from *H. erinaceus* exhibited the most potent bactericidal activity against *S. aureus* (Nikolovska-Nedelkoska *et al.* 2017). The presence of antimicrobial activity was also detected in the aqueous extracts of *H. coralloides* fruiting bodies against *E. coli*, *M. luteus* and *B. subtilis,* while the ethanol extracts only inhibited the growth of *B. subtilis* (Pasaylyuk 2017). The inhibited growth of *E. coli* and *S. aureus* was demonstrated by the *H. americanum* mycelium, while the mycelium of *H. erinaceus* only inhibited the growth of *S. aureus* (Julian *et al.* 2018). Extracts from mycelial cultures of a unique North American *Hericium* sp. inhibited the growth of *C. albicans* and *C. neoformans* (Song *et*

al. 2020). In general, the antibacterial activity of *Hericium* species varied from low (8 to 12 mm) to high (28 to 32 mm) according to the aforementioned investigations.

The obtained results indicate the ability of the studied *Hericium* species to produce antibiotic metabolites with both a wide and narrow spectrum of action. Our results confirm the importance of investigating the antibacterial activity of different species and strains. To the best of our knowledge, *H. abietis* and *H. cirrhatum* species are quite rare and thus have never been studied for antibacterial activity. Furthermore, as far as we know, the ability of *H. coralloides* to inhibit the growth of *P. aeruginosa* and *S. aureus* has not been reported previously.

In our study the activity of the strains varied significantly, whereby the zone of inhibition of the tested bacteria was in the range of 5.3 mm to 20.0 mm (Tables 2 & 3). In some cases, antibacterial activity was observed only after culture filtrate concentration. This tendency was demonstrated for those strains with very low antibacterial activity (*H. abietis* IBK 2376, *H. coralloides* IBK 1876, 2333, and *H. erinaceus* 339, 965, 2239). Improving the antibacterial activity of the culture filtrate by means of increased concentration has also been described in previous studies (Efremenkova *et al*. 2001; Krupodorova *et al*. 2019; Mykchaylova & Poyedinok 2021).

All these observations comply with previously reported data confirming the antibacterial activity of *H. erinaceus* against *B. subtilis* (Okamoto *et al*. 1993; Wong *et al*. 2009; Han *et al*. 2015; Krupodorova *et al*. 2016; Lomberg 2021), *E. coli*, *M. luteus*, and *P. aeruginosa* (Wong *et al*. 2009; Han *et al*. 2015), and *S. aureus* (Kim *et al*. 2000; Kawagishi 2005; Wong *et al*. 2009; Han *et al*. 2015; Krupodorova *et al*. 2016; Nikolovska-Nedelkoska *et al*. 2017; Julian *et al*. 2018), in contrast to other data showing the absence of *E. coli* growth inhibition (Krupodorova *et al*. 2016; Julian *et al*. 2018; Lomberg 2021). The activity of the studied *H. erinaceus* strains against *B. subtilis*, *E. coli*, and *P. aeruginosa* was higher than that observed by Han *et al*. (2015), but lower compared to the results obtained by Wong *et al*. (2009). The inhibition of *M. luteus* growth was similar in some examples, but was mainly significantly higher than reported by Han *et al*. (2015). *S. aureus* growth inhibition was higher than that stated by Han *et al*. (2015), in line with some other reports (Krupodorova *et al*. 2016; Julian *et al*. 2018), but far lower than observed by Wong *et al*. (2009).

The antibacterial activity of *H. coralloides* against *B. subtilis*, *E. coli*, and *M. luteus* corresponds to the results gained by Pasaylyuk (2017), however our strain 2332 demonstrated significantly higher activity. Differences in the results may be due to the intraspecific genetic peculiarity of the species as well as the strains of the fungus and tested bacteria, extract preparation and the period of evaluation and interpretation of the results.

Determining the duration of the cultivation of the culture with the highest antibacterial activity is one of the important steps in optimising the biotechnological cultivation protocol. According to our results, the cultivation period with the best antibacterial activity (7 or 14 days) was strain-dependent on the fungal culture from the genus *Hericium* as well as the tested bacterium. This observation complies with other reports (Krupodorova *et al*. 2008; Sazanova *et al*. 2013; Krupodorova *et al*. 2020; Metreveli *et al*. 2021; Mykchaylova & Poyedinok 2021) which stated that the manifestation of antimicrobial activity of different fungi may be contingent upon various stages of fungi development. Presumably, it may be due to the different nature of the active secondary metabolites produced by fungi in these stages or/and their concentration.

Approximately 83 bioactive compounds belonging to terpenoids, phenolics, fatty acids, steroids, and alkaloids have been detected in the mycelia and the fruiting bodies of *Hericium* species (Wang *et al*. 2015). Less information is available about the main biologically active substances of culture filtrates from the genus *Hericium* (namely, *Hericium erinaceus*) such as erinapyrones A, B (Kawagishi *et al*. 1992), erinacine P, Q (Kenmoku *et al*. 2002), and erinaceolactones A to C (Wu *et al*. 2015). Although a fair number of compounds have been isolated from *Hericium* species, not enough research has been focused on the substances responsible for the antibiotic action of mycelium and culture filtrates. Chlorinated orcinol derivatives such as $C_9H_{11}O_2Cl$, $C_9H_{11}O_3Cl$, $C_9H_9O_3Cl$ isolated from *H. erinaceus* mycelium inhibited the growth of *Bacillus subtilis* (Okamoto *et al*. 1993). Erinapyrone C extracted from the mycelium of *H. erinaceus* exhibited moderate activity against Gram-positive bacteria (Arnone *et al*. 1994). Erinacines A, B, K were isolated as anti-MRSA compounds from the *H. erinaceus* cultured mycelia (Kawagishi *et al*. 1994, 2006; Kawagishi 2005). Presumably, the antibacterial substances mentioned above may also be responsible for the activity in our studied *Hericium* species.

CONCLUSION

The obtained results confirmed and expanded our knowledge of the antibacterial potential of *Hericium* species and pointed to the relevance and importance of this line of research. To the best of our knowledge, the antibacterial activity of *H. abietis* and *H. cirrhatum* species was established for the first time. As far as we are aware, the ability of *H. coralloides* to inhibit the growth of *P. aeruginosa* and *S. aureus* has also not been previously reported. Our findings enable us to conclude that the studied *Hericium* species and strains produce active antibacterial metabolites. Further studies are required for the isolation and determination of the biologically active substances responsible for their antimicrobial properties, which could be regarded as potential sources of health benefits.

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REZIME

Botanica SERBICA

Antibakterijska aktivnost filtrata kulture i micelija odabranih sojeva makromiceta iz roda *Hericium*

Margarita Lomberg, Tetiana Krupodorova, Viktoriia Krasinko i Oksana Mykchaylova

Cilj rada bio je da se ispita antibakterijska aktivnost sojeva roda *Hericium*, koji pripadaju bazidiomicetima, iz IBK Zbirke kulture gljiva M.G. Instituta za botaniku Holodnog Nacionalne akademije nauka Ukrajine. Ispitivano je ukupno 14 sojeva uključujući *H. abietis*, *H. cirrhatum*, *H. coralloides* i *H. erinaceus*. Sojevi su kultivisani na tečnom medijumu glukoza-pepton-kvasac. I homogenizovani micelijum i filtrat ovih gljiva su procenjeni na gram-pozitivne (*Bacillus subtilis*, *Micrococcus luteus*, *Staphilococcus aureus*) i gram-negativne (*Escherichia coli*, *Pseudomonas aeruginosa*) bakterije metodom disk difuzije i cilindara. Aktivnost sojeva je značajno varirala. U zavisnosti od tehnike ispitivanja, vrste, soja i stadijuma razvoja gljivice, zona inhibicije ispitivanih bakterija se kretala od 5,3 mm do 20,0 mm. Generalno, antibakterijski potencijal filtrata kulture ispitivanih vrsta bio je značajno veći od njihove micelijske aktivnosti. Homogenizovani micelijum je pokazao potencijalno dobre rezultate samo protiv *E. coli*. Prvi put su uočene antibakterijske aktivnosti vrsta *H. abietis* i *H. cirrhatum*. Koliko nam je poznato, sposobnost *H. coralloides* da inhibira rast *P. aeruginosa* i *S. aureus* nije ranije prijavljivana. Dobijeni rezultati su ukazali na sposobnost proučavane vrste *Hericium* da proizvodi antibakterijske metabolite širokog i uskog spektra delovanja koji mogu imati potencijalne zdravstvene koristi i mogu se preporučiti za dalju analizu, izolaciju i identifikaciju antibakterijskih jedinjenja potencijalno perspektivnih u farmakologiji.

Ključne reči: antibakterijsko dejstvo, bazidiomicete, filtrat kulture, micelijum.