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RESEARCH ARTICLE

Antimicrobial activity in cultures of some xylotrophic basidiomycetes from Azerbaijan

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Abstract. Antimicrobial activity of several strains of *Laetiporus sulphureus*, *Phellinus igniarius*, and *Pleurotus ostreatus* against Gram-positive and Gram-negative bacteria *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* was studied. It has been demonstrated that under the influence of ethyl acetate extracts of the culture liquid of *L. sulphureus* 2780, the inhibition zones of growth of all investigated species of bacteria varied within 7.5–12.0 mm. The extracts of the biomass and culture fluid of *L. sulphureus* 2780, *Phellinus igniarius* 2781, and *Pleurotus ostreatus* 2779 inhibited the growth of *Staphylococcus aureus* (inhibition zones — 7.5–10.0 mm) and *Bacillus subtilis* (inhibition zones — 9.5–12.5 mm). The results indicate the potential of the strains *L. sulphureus* 2780, *Ph. igniarius* 2781, and *P. ostreatus* 2779 from the IBK Mushroom Culture Collection as antimicrobial agents against the Gram-positive bacterium *Bacillus subtilis*.

Keywords: antibacterial activity, biomass, cultural fluid, *Laetiporus sulphureus*, *Phellinus igniarius*, *Pleurotus ostreatus*

Introduction

The widespread, and often unjustified, use of antibiotics is known to contribute to the emergence and dissemination of drug-resistant strains of pathogenic microorganisms (Theuretzbacher, 2013; Urban-Chmiel et al., 2022; Ho et al., 2025, etc.). The growing resistance of these pathogens to existing treatments underlines the urgent need to identify new effective producers of natural antibiotics or other antibacterial agents. Recently, the interest of researchers in higher basidiomycetes as potential sources of biologically active substances, including antibiotics, has increased. The literature provides the reports on the antimicrobial activity of extracts derived from fruiting bodies, mycelial biomass, and cultural fluids of various species of higher basidiomycetes. In particular, many species of basidial xylotrophic mushrooms have been found to produce a complex of biologically active substances with

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antimicrobial properties (Krupodorova et al., 2016, 2019; Mykchaylova, Poyedinok, 2021; Mustafin et al., 2022; Vazquez-Armenta et al., 2022; Agarwal et al., 2023; Narmuratova et al., 2023).

Notably, a significant portion of the published data is focused on the antimicrobial activity of various types of extracts obtained from macrofungal fruiting bodies. For example, the ethanol extracts of basidiocarps of *Laetiporus sulphureus* (Bull.) Murrill have been shown to possess a moderate antimicrobial activity against both Gram-positive bacteria *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, and *Micrococcus luteus*, as well as Gram-negative bacteria *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Pseudomonas mirabilis* (Nowacka et al., 2014). However, some authors did not report any activity of such extracts against *Klebsiella pneumoniae* (Turkoglu et al., 2007). In the other research, aqueous extracts of *Laetiporus sulphureus* fruiting bodies exposed activity only against Gram-positive *Micrococcus flavus* and *Listeria monocytogenes* (Šiljegović et al., 2011). Similarly, it was found that ethanol, aqueous, acetone, ethyl acetate, and chloroform extracts showed inhibition of the growth of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (Younis et al., 2019).

The ethanol and ethyl acetate extracts of fruiting bodies of *Pleurotus ostreatus* (Jacq.) P. Kumm. were reported as a source of antibacterial activity against Gram-positive bacteria *Bacillus cereus* and *Staphylococcus aureus,* as well as Gram-negative bacteria *Escherichia coli*, *Escherichia cloacae*, *Pseudomonas mirabilis*, *Pseudomonas aeruginosa*, *Serratia marcescens*, and *Salmonella typhi* (Sutthisa, Anujakkawan, 2023). Moreover, it was demonstrated that antibacterial activity of the investigated microorganisms depended on the chosen solvent. The ethanol extracts exhibited noteworthy inhibitory effects on the growth of *Bacillus cereus*, *Escherichia cloacae*, *Pseudomonas aeruginosa*, *Serratia marcescens*, and *Salmonella typhi.* At the same time, ethyl acetate extract exposed antimicrobial activity only against *Escherichia coli* (Sutthisa, Anujakkawan, 2023). Other authors reported antimicrobial activity of the ethyl acetate extracts of fruiting bodies of *Pleurotus ostreatus* against *Escherichia coli* and *Bacillus subtilis* (Agarwal et al., 2023), while, according to the other report, the water extract of basidiocarps of the same fungus exhibited antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Bawadekji et al., 2017).

Based on the specimens deposited at the Mycological Herbarium (BAK) of the Institute of botany MSERA, 827 species of mushrooms belonging to 210 genera are currently reported in Azerbaijan (Sadiqov, Aghaeva, 2016; Mustafabayli et al., 2021). Of them, *Laetiporus sulphureus*, *Phellinus igniarius* (L.) Quèl., and *Pleurotus ostreatus* are xylotrophic macromycetes commonly occurring in Azerbaijan, including Guba District.

Our research aimed to investigate the antimicrobial activity of biomass and culture fluid extracts of strains of *Laetiporus sulphureus*, *Pleurotus ostreatus*, and *Phellinus igniarius* against Gram-negative and Gram-positive bacterial species.

Materials and Methods

Mushroom strains and cultivation conditions. Three mushroom strains (*Laetiporus sulphureus* 2780, *Phellinus igniarius* 2781, and *Pleurotus ostreatus* 2779) were isolated in pure culture from carpophores growing on deciduous tree species in Guba District, Azerbaijan, in the autumns of 2019–2020 (Fig. 1). These strains were deposited in the IBK Mushroom Culture Collection of the M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine (Bisko et al., 2023), and used for further experiments.

Mycelial cultures were grown in Petri dishes (90 mm diam.) on glucose-peptone-yeast agar medium (GPYA), composed of, g/l: glucose 24.0, peptone 2.0, yeast extract 3.0, K_2HPO_4 1.0, KH_2PO_4 1.0, $MgSO_4 \times 7H_2O$ 0.25, agar 20, distilled water 1l (pH 6.0), at the temperature 27 ± 0.1 °C. The medium was sterilized by autoclaving for 20 min at 121 °C. Mycelia of the studied strains in the active growth stage (5 disks 7 mm diam.) were used as an inoculum which was inoculated in 250 ml Erlenmeyer flasks, containing 50 ml glucose-peptone-yeast medium (GPY). The mushroom strains were cultivated by the submerged method to obtain mycelial biomass and culture liquid for 7 days at the temperature of 27 ± 0.1 °C. The biomass was separated from the culture liquid through a nylon filter, dried at 60 °C for constant weight, and grinded to a powdery state.

Preparation of biomass and culture liquid extracts. To obtain the extract of the biomass using ethyl acetate as a selected solvent (Agarwal et al., 2023), the ethyl acetate was added to biomass in a

Fig. 1. Fruiting bodies of the studied mushroom species used for isolation. A: *Laetiporus sulphureus* 2780; B: *Phellinus igniarius* 2781; C: *Pleurotus ostreatus* 2779

ratio of 2 : 1 (v/w) and incubated for 7 days at the temperature of 27 ± 0.1 °C on a rotary shaker (150 rpm, darkness). Then this mixture was centrifuged. The clear supernatant was stored at 5.0 ± 0.1 °C in the refrigerator for further analysis.

To obtain an ethyl acetate extract of the culture liquid, ethyl acetate in a ratio of 2 : 1 (v/v) was added to culture liquid and incubated in the refrigerator in darkness at the temperature of 5.0 ± 0.1 °C for 24 hours. Then the ethyl acetate was removed, evaporated using a vacuum evaporator. The dry residue was dissolved in methanol. The extract was stored at 5.0 ± 0.1 °C for further study.

Test bacterial cultures. The cultures of two species of Gram-positive bacteria, *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 65388, and three species of Gram-negative bacteria, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* M 123, and *Pseudomonas aeruginosa* ATCC 27833, from the Ukrainian Collection of Microorganisms, UCM (D.K. Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine) were used. Bacterial cultures were grown in tubes on the Mueller-Hinton agar medium (Oxoid). The bacterial colony of each test culture was transferred into a tube containing sterile water, shaken to obtain a homogeneous suspension, bringing the inoculum density to 5 × 10 cells/ml. A suspension of bacteria from these tubes (0.2 ml) was spread on the surface of the Mueller-Hinton agar medium in Petri dishes. Extracts of the biomass and the culture liquid (10 µl) were applied to standard bioMérieux firm discs (6 mm diam.), dried at 35–40 °C for 30 min and placed on the surface of the Mueller-Hinton agar medium in Petri dishes pre-inoculated with the investigated bacterial cultures. After that, Petri dishes were incubated at 37 ± 0.1 °C for 24 hours. Antibacterial activity was measured in terms of inhibition zone size (in mm) around discs. Antibiotic gentamicin sulfate (4 mg/ml, Ukraine) was used as a positive control. The ethyl acetate was used as a negative control.

Table 1. **Antibacterial activity of ethyl acetate extracts of biomass and cultural liquid of *Laetiporus sulphureus* 2780, *Phellinus igniarius* 2781, and *Pleurotus ostreatus* 2779 ***

Test bacteria	Inhibition zone (mm) (Mean ± SD)					
	Laetiporus sulphureus		*Phellinus igniarius*		*Pleurotus ostreatus*	
	Culture liquid	Biomass	Culture liquid	Biomass	Culture liquid	Biomass
Escherichia coli	8.5 ± 0.3	–	7.5 ± 0.1	7.5 ± 0.2	–	–
Staphylococcus aureus	7.5 ± 0.2	8.0 ± 0.2	7.5 ± 0.1	7.5 ± 0.1	10.0 ± 0.2	8.5 ± 0.2
Klebsiella pneumoniae	9.0 ± 0.1	–	–	–	8.0 ± 0.2	–
Bacillus subtilis	12.0 ± 0.2	12 ± 0.2	12.5 ± 0.3	9.5 ± 0.1	12.2 ± 0.2	10.0 ± 0.2
Pseudomonas aeruginosa	9.0 ± 0.1	9.0 ± 0.2	8.0 ± 0.1	9.0 ± 0.1	–	8.0 ± 0.1

* Positive control — gentamicin sulfate — inhibition zone 18.5 ± 0.3 mm; negative control — ethyl acetate — inhibition zone 0; "–" no antibacterial activity.

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 recorded as means ± standard deviation using Microsoft Excel 2010.

Results and Discussion

As seen in Table 1, the ethyl acetate extracts obtained from both the biomass and the culture liquid of the studied mushroom strains showed some activity against Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis*. Nevertheless, inhibition zones toward *Bacillus subtilis* were larger than those in case of *Staphylococcus aureus* (Table 1). The tested strains of macromycetes exhibited distinct levels of activity also against Gram-negative bacteria. Both the culture liquid and biomass extracts of *L. sulphureus* and *Ph. igniarius* inhibited the growth of *Pseudomonas aeruginosa*. At the same time, antimicrobial activity against *Klebsiella pneumoniae* was observed only for extracts of culture liquids of *L. sulphureus* and *P. ostreatus*. Our results demonstrated that all investigated mushroom species were the most active against *Bacillus subtilis* (Table 1). The ethyl acetate extracts obtained both from the culture liquid and biomass of *Ph. igniarius* were not inhibitory against *Klebsiella pneumoniae*. Similarly, ethyl acetate extracts obtained both from culture liquid and biomass of *P. ostreatus* had no inhibitory effect against *Escherichia coli.* Overall, inhibition zones produced by all mushroom strains were smaller as compared to those of the antibiotic gentamicin sulfate (Table 1).

Previously published data reported that the mycelial mass and culture liquid extracts of other nine strains of *L. sulphureus* from the IBK Mushroom Culture Collection of the M.G. Kholodny Institute of Botany possessed some activity against *Pseudomonas aeruginosa* (Dzyhun et al., 2011). For three of those strains, antimicrobial activity against *Staphylococcus aureus* was also detected for biomass extracts, as well as for a culture liquid extract for one of the strains. In the case of *Escherichia coli*, such activity was observed for culture liquids of three strains and for the mycelial mass of one strain. Of nine strains of *L. sulphureus*, a single strain only exposed some activity against *Bacillus subtilis* (Dzyhun et al., 2011). Recently, the antimicrobial activity of *L. sulphureus* biomass against *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus* was reported (Nicolcioiu et al., 2017).

Thus, the obtained results indicate that the studied strains *L. sulphureus* 2780, *P. ostreatus* 2779, and *Ph. igniarius* 2781, three widespread species of xylotrophic fungi in forests of Azerbaijan, possess antimicrobial activity against both Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*).

Conclusion

Our research demonstrated that the ethyl acetate extract of the culture liquid of *L. sulphureus* 2780 exhibited the widest spectrum of antimicrobial activity against all studied microorganisms. The extract of culture liquids and biomass of *L. sulphureus* 2780, *Ph. igniarius* 2781, and *P. ostreatus* 2779 proved to be highly active against *Bacillus subtilis.* The greatest growth inhibition effect for *Staphylococcus aureus* was shown by the extract of culture fluid of *P. ostreatus* 2779. These promising results also indicate that further studies on these and other selected

strains of fungi are needed for searching for efficient fungal antimicrobial agents, including species of fungi occurring in Azerbaijan.

ETHICS DECLARATION

The authors declare no conflict of interest.

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Антимікробна активність культур деяких ксилотрофних базидіоміцетів з Азербайджану

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Реферат. Досліджено антимікробну активність культур *Laetiporus sulphureus*, *Phellinus igniarius* і *Pleurotus ostreatus* проти грам-позитивних та грам-негативних штамів *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* та *Pseudomonas aeruginosa*. Встановлено, що під впливом етилацетатних екстрактів культуральної рідини штаму *L. sulphureus* 2780 зони інгібування росту всіх досліджених видів бактерій становили 7,5–12,0 мм. Як екстракти біомаси, так і культуральної рідини *L. sulphureus* 2780, *Phellinus igniarius* 2781 та *Pleurotus ostreatus* 2779 пригнічували ріст *Staphylococcus aureus* (зони інгібування — 7,5–10,0 мм) і *Bacillus subtilis* (зони інгібування — 9,5–12,5 мм). Отримані результати свідчать про перспективність використання штамів *L. sulphureus* 2780, *Ph. igniarius* 2781 та *P. ostreatus* 2779 з ІВК Колекції культур шапинкових грибів як антимікробних агентів проти грам-позитивної бактерії *Bacillus subtilis*.

Ключові слова: *Laetiporus sulphureus*, *Phellinus igniarius*, *Pleurotus ostreatus*, антибактеріальна активність, біомаса, культуральна рідина