

Biotechnological potential of xylotrophic macromycetes distributed in Azerbaijan as producers of polysaccharides

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Abstract

The purpose of the presented work was to determine the species composition of the mycobiota of xylotrophic macromycetes distributed on trees in the territory of the Republic of Azerbaijan, the creation of a collection consisting of cultures of the species participating in its formation, and the biotechnological potential of the species belonging to the genus *Ganoderma* included in the collection as producers of polysaccharides. It has become clear that 72 species belonging to 40 genera participate in the formation of the xyломicobiota of trees distributed in the conditions of Azerbaijan, 4 of which belong to the genus *Ganoderma*. Although species belonging to the genus *Ganoderma* differ from one another in terms of growth rates, the amount of polysaccharides they produce, as well as their quality indicators, strains belonging to *G. applanatum* and *G. lucidum* differed from the others in all indicators. This difference is reflected in both the quantitative and qualitative characteristics of the polysaccharides they produce. Thus, the total amount of polysaccharides they formed was 1.4-2.2 times higher than that of others, and the share of 1-3- β -glucosidic bonds in their composition was relatively high. This is a serious basis for their use for practical purposes in the future.

Keywords: Xylomycobiota, species composition, collection, *Ganoderma* genus, polysaccharide, active producer, β -glucosidic bonds

1. Introduction

The increasingly significant changes in the conditions in which people live due to the influence of physical, chemical, and biological factors have led to changes in their adaptive capacity and

resistance, and this process continues to this day. Thus, the influence of the mentioned factors, which are mainly characterized by negative aspects, is still developing with a rising line, and no one doubts that this will continue at least in the future. For this reason, the trend of deteriorating human health, expanding the geography of existing diseases, and the emergence of new pathogens is clearly felt everywhere on Earth. For this reason, the prevention of the aforementioned, the development of new and more effective drugs for the treatment of diseases, as well as the acquisition of functionally active food supplements and medical drugs that serve to strengthen the human immune system, are important research directions in the modern era and are becoming increasingly relevant. Therefore, the prevention of the above-mentioned, the development of new and more effective drugs for the treatment of diseases, as well as the acquisition of functionally active food supplements and medical drugs that serve to strengthen the human immune system and others are among the current research directions that are important to address in the modern era (Geng *et al.*, 2025, Gottdenker *et al.*, 2014), which is becoming even more relevant in the modern era (Shivanna, 2022) when global environmental problems are felt. One of the reasons why this issue is relevant is that the effectiveness of drugs used to treat certain diseases, especially those obtained through chemical synthesis, decreases after a certain time, meaning that resistance develops (Salam *et al.*, 2023).

To solve the above-mentioned problems, today the most promising option is to obtain the necessary products from bioresources, including from mushrooms. Fungi are a relatively small group of eukaryotic organisms known to science, so the number of fungal species currently known to science is around 150-170 thousand, which is less than the number of known plant (400,000 species) and animal (1.5 million) species (Michael, 2024). However, the actual number of mushrooms in nature is many times greater, and the ecological functions they perform in nature are not only broader and irreplaceable but also more critical than those of other taxa (Cazabonne *et al.*, 2024, Jie *et al.*, 2024, Phukhamsakda *et al.*, 2022). It should only be noted that there is no function in nature in which mushrooms do not participate. The practical interest in mushrooms is based on the wide and diverse functions they perform in nature. For this reason, the possibilities of using mushrooms are one of the most widely studied and relevant research areas today (Bell *et al.*, 2022, Bhambri *et al.*, 2022). Thus, the results of studies conducted in a number of scientific centers have determined that the use of mushrooms for practical purposes is more favorable than that of bacteria, plants, and animals (Corbu *et al.*, 2023, Pouris *et al.*, 2024).

Due to the large number of actual species in nature and the importance of the functions they perform, mushrooms are grouped not only from a taxonomic perspective but also according to various characteristics, one of which is based on their ecological indicators. One of those that fit this characteristic is xylotrophs. Xylotrophic mushrooms, especially those species that cause white rot in natural conditions, can also assimilate polysaccharides (pentoses, galactose, hemicellulose, cellulose, lignin, etc.) that are not of nutritional value and have varying degrees of polymerization (Qi *et al.*, 2023). This creates opportunities for the utilization of raw materials with various non-food properties, especially lignocellulose components, which are formed in large quantities every year after the use of green biomass formed as a result of the photosynthesis process, by using them for cultivation (Huang *et al.*, 2023). In short, basidiomycetes, especially their xylotrophic species, are an open and interesting object of research today.

There is no doubt that the diversity of Azerbaijan's nature, the richness of its flora and fauna, and the wide variety of climatic and soil conditions in a place that occupies a small area also contribute to the diversity of mushrooms found in these areas. This is confirmed by a number of studies, as it is clear from the studies that representatives of all taxonomic groups of mushrooms that exist today are widespread in the territory of Azerbaijan. Among them are xylotrophic macromycetes, and there is literature information that their number is 214 (Bakshaliyeva *et al.*, 2023a). A lot of research has been conducted (Bakshaliyeva *et al.*, 2020,

Shatirova *et al.*, 2022) on the study of these mushrooms as producers of biologically active substances (BAS), and in this aspect, their study as producers of enzymes (Akhundova *et al.*, 2019, Bakhshaliyeva *et al.*, 2023) is more extensive. The number of species of xylophilic macromycetes studied for their fermentative activity is relatively higher, and the xylophilic macromycetes involved in the screening process constitute a small part of the total xylophilic macromycetes distributed in Azerbaijan. The number of xylophilic macromycetes studied in Azerbaijan as producers of other types of BAS, especially polysaccharides with functional activity, is quite small. Considering that the quantitative indicator of the characteristic properties of a particular fungus is unstable even at the strain level, and that its formation is influenced by the climatic, soil, and other conditions of the area where the fungus is distributed, studying xylophilic macromycetes in a specific region from this aspect can be considered an approach that retains its importance.

Taking into account the above, the aim of the presented work was to create a collection of cultures of macromycetes participating in the formation of the xylomycobiota of trees distributed in various areas of the Republic of Azerbaijan and to evaluate the biotechnological potential of some mushrooms included in the collection as producers of polysaccharides.

2. Materials and Methods

Samples for the studies were taken from trees growing wild or cultivated in various areas of the Republic of Azerbaijan (natural and artificial forests, parks and gardens, green spaces). The samples were taken from the main geomorphological units of the Republic of Azerbaijan (38° 24' - 41° 54' N and 44° 46' vø 50° 50' E), which are mainly forests (parts of the Greater and Lesser Caucasus belonging to the territory of Azerbaijan, including the Karabakh and Northern Zangezur Economic Regions (ER), the Kur-Araz Lowland (Central Aran ER), and the Lankaran-Astara ER (Fig. 1). Sampling, more precisely, the collection of basidiomas belonging to xylophilic macromycetes, was carried out along a route selected for a specific area.

As a source of biologically active substances (BAS), the 5-day biomass (BM) and culture medium (CM) of the mushroom grown in the vegetative growth phase in LGPM were used. In order to separate exopolysaccharides from the CM, the solution obtained after filtration was concentrated several times using a vacuum rotor evaporator. The solid solution is treated with 96% ethyl alcohol in a ratio of 1:2, and the resulting precipitate (by centrifugation for 15 min, 5000 rpm) is dried, powdered, and analyzed as an exopolysaccharide fraction (Miao *et al.*, 2020). The separation of the polysaccharide fraction from the mycelium was carried out according to the method used in the work of various authors (Do *et al.* 2020, Liu *et al.*, 2022). When determining the qualitative and quantitative composition of polysaccharide fractions obtained from mushrooms, methods based on the complete hydrolysis process with the help of acids and gas-liquid chromatography were used (Guo *et al.* 2019, Shukla *et al.*, 2022).

Pure cultures were obtained from basidiomas collected in the laboratory using classical methods and approaches applied in mycological research (Maheshwari, 2016). Agarized malt juice (AMJ, 2-3^oB) was used to obtain pure culture, and the purity of the culture was monitored using a microscope (OMAX 40X-2500X LED Digital Lab Trinocular Compound Microscope). The process of obtaining vegetative mycelium from mushrooms was carried out in the liquid glucose-peptone medium (LGPM) used in our previous work (Bakhshaliyeva *et al.*, 2020).



Figure 1. General view of the research areas (●)

The total protein content in BM and CM was determined using Lowry's assay. An aliquot of 1 mL of extract was treated with 5 mL of Reagent A and kept in the dark for 10 min. Then, the mixture was loaded with 0.5 mL of Folin-Ciocalteu reagent and left in a dark place for 30 min. After incubation, the absorbance was measured at 630 nm. Protein concentration (mg/mL) was determined using the Bovine Serum Albumin (BSA) standard curve in the extracted samples. Reagent A is made by combining 99 mL of reagent B (2% Na_2CO_3 and 0.1N NaOH), 0.5 mL of reagent C (2% of copper sulfate pentahydrate), and 0.5 mL of reagent D (2% of potassium sodium tartrate) (Srivastava et al., 2024). The antimicrobial activity of polysaccharide fractions obtained from the studied mushrooms was carried out using a slightly modified disk-diffusion method [Gajic et al., 2022] using traditional test cultures. The essence of the change was as follows: the amount of the studied substance was 100 μl , the concentration of test cultures was 10^7 CFU/ml, the nutrient medium used for the cultivation of bacteria was Müller-Hinton, Saburo agar, for positive control in bacteria – Ciprofloxacin (5 μg /well), for mushrooms – fluconazole (5 μg /well), and as a negative control, 3% dimethylsulfoxide was used for both microorganisms. The minimum inhibitory concentration of the studied substances was carried out according to the protocol used in the work of Chowdhury et al. (2015).

All experiments in the studies were performed in at least 4 replicates. The results were statistically analyzed using STATISTICA version 13.0 software (TIBCO Software Inc., Palo Alto, CA, USA) with ANOVA models and multiple Tukey's t-tests at the significance level of $\alpha = 0.05$.

3. Results

During the research, the species composition of the xylomycobiota of trees in the territories of Azerbaijan, differing in natural soil and climate conditions and flora, was determined for

the first time, and issues related to the creation of a collection of their strains were resolved. In this regard, 730 fruiting bodies of xylotrophic macromycetes were collected from the research areas in 2016-2024, and their species composition was determined by classical mycological methods. It became clear that they belonged to a total of 72 species, such as *Abortiporus bienus* (3 strains), *Armillaria gallica* (3), *A.mellea* (4), *Bjerkandera adusta* (4), *B.fumosa* (3), *Cerrena unicolor* (2), *Climacodon pulcherrimus* (2), *Daedalea quersina* (2), *Daedaleopsis confragosa* (3), *Flammulina velutipes* (3), *Fomes fomentarius* (5), *Fomitopsis cyticina* (4), *F.officialis* (4), *F.pinicola* (6), *F.hartigii* (4), *F.contigua* (2), *F.torulosa* (3), *Ganoderma adspersum* (5), *G.applanatum* (= *G.lipsiense*) (14), *G.lucidum* (11), *G.resinaceum* (6), *Heterobasidium annozum* (3), *Hyphoderma setigerum* (4), *Inonotus cuticularis* (1), *I.hispidus* (4), *I.pini* (2), *I.radiatus* (1), *Laetiporus sulphureus* (5), *Lentinus sitrogosus* (3), *L.tigrinus* (3), *Lenzites betulina* (4), *Marasmius alliaceus* (2), *M.androsaceus* (2), *M.epiphyllus* (3), *M.oreades* (2), *M.rotula* (2), *Peniophora gigantea* (3), *Phaeolus schweinitzii* (4), *Phellinus contiguus* (4), *Ph.gilvus* (5), *Ph.igniarius* (8), *Ph.pini* (4), *Ph.pomaceus* (5), *Ph.robustus* (5), *Ph.tremulae* (4), *Ph.populnea* (4), *Pleurotus cornucopiae* (3), *P.ostreatus* (8), *P.pulmonarius* (3), *Pluteus cervinus* (3), *P.leoninus* (5), *P.plautus* (3), *Polyporus squamosus* (5), *P.vaporarius* (4), *P.varius* (3), *Porodaedalea chrysoloma* (4), *Pycnoporus cinnabarinus* (3), *Rigidoporus ulmarius* (4), *Rhodoformes rosesus* (5), *Pseudoinonotus dryadeus* (3), *Schizophyllum commune* (5), *Stereum gausapatum* (3), *S. hirsutum* (4), *Trametes heteromorpha* (2), *T.hirsuta* (7), *T.ochracea* (3), *T.pubescens* (4), *T. versicolor* (8), *T. zonata* (3), *Trichaptum bifforme* (3), *Tyromyces amorphus* (3), *Vuilleminia comedens* (3) whose distribution in individual genera was also different.

As seen, although no genus with a maximum number of species exceeding 10 has been found, 7 species belonging to the genus *Phellinus* and 5 to the genus *Trametes* have been recorded in studies. The number of species belonging to the remaining genera varies between 1-4.

It should be noted that biomass from mushrooms can be obtained in any quantity and at any time of the year during the vegetative phase (VF) of growth. Targeted products, including BAS from mushrooms, are produced in both FB and VF, so it is considered appropriate (Gracheva *et al.*, 2021) to create a collection of strains specific to such mushroom species, and this issue has been positively resolved in our research. Thus, during the research, a collection of 277 strains was created.

Based on the research materials, it was established that many of the species to which the strains in the collection belong are promising as producers of certain BASs. Interestingly, the BASs synthesized by mushrooms belonging to the same species but isolated from different ecological conditions differ from each other, at least quantitatively. This allows us to note that the possibilities of finding a more active natural strain in specific conditions have not been exhausted and that research in this direction is relevant. For this reason, in the next stage of research, strains belonging to a specific genus were individually evaluated as BAS producers, which was first started with the strains belonging to the genus *Ganoderma*. It should be noted that one of the main indicators for using microorganisms, including mushrooms, as BAS producers is that the selected producer produces more biomass in a short period of time. For this reason, for the above-mentioned purposes, especially in studies conducted during the vegetative phase of growth, the initial screening of cultures is carried out from this aspect, and in the presented study, the initial selection or screening of mushrooms belonging to the genus *Ganoderma* was carried out from this perspective. It became clear that the tested strains differ according to this indicator, and among them are both promising ones, that is, those with a high growth rate (those that produce more than 5 g/l of biomass in 7 days), and those that are not suitable (those that produce less than 5 g/l of biomass in 7 days) for use today due to biomass yield. Although strains with rapid growth capacity accounted for 27.1% of those tested, almost all of them belonged to two species (*G. applanatum* and *G. lucidum*) (Table

1). The selection of *G. applanatum* S-69 (9.6 g/l biomass) and *G. lucidum* S-76 (9.3 g/l biomass), which differ from all strains in terms of biomass yield, as active producers is a logical result of the studies conducted at this stage.

A characteristic of any organism is associated with its genome, but nevertheless, the physicochemical, or more precisely, ecological factors of the environment also play a certain role in its emergence, and the correct determination of these factors allows for maximum results, and this has been repeatedly confirmed in studies conducted by various authors. It is clear from these studies that when the authors say environmental parameters, they primarily mean carbon and nitrogen sources, cultivation temperature, initial acidity, storage of planting material, and other factors.

Taking into account the above, the research also included studies on the optimization of these parameters for the strains selected as active producers. According to the obtained results, the amount of carbon source, i.e. glucose added to the medium is 9.5-9.7 g/l, organic nitrogen source, i.e. peptone 3.0 g/l, mineral nitrogen source, i.e. NH_4NO_3 - 1.5 g/l (more precisely, 0.037-0.038% for nitrogen), cultivation temperature 28°C, initial acidity of the medium, i.e. pH=5.5 is favorable.

Table 1 Characterization of *Ganoderma* strains according to the amount of biomass they produce (7 days, DC)

| № | Species | Total number of strains | Amount of biomass produced, g/l | | |
|-------|----------------------|-------------------------|---------------------------------|----------------|--------------|
| | | | Fast growing (number/biomass) | Medium growing | Late-growing |
| 1 | <i>G. adspersum</i> | 8 | 1/5,4 | 4/3,2-4,6 | 3/1,2-2,7 |
| 2 | <i>G. applanatum</i> | 11 | 5/6,7-9,6 | 2/3,1-4,8 | 4/1,5-2,9 |
| 3 | <i>G. lucidum</i> | 10 | 4/6,2-9,3 | 2/3,6-4,5 | 4/1,3-2,1 |
| 4 | <i>G. resinaceum</i> | 7 | 1/5,8 | 3/3,3-4,6 | 3/1,1-2,8 |
| Total | | 36 | 11(5,4-7,5) | 14(1,1-2,9) | 11(3,1-4,8) |

As mentioned, in most studies, environmental optimization is usually carried out according to the same parameters, but sometimes it is possible to achieve a certain effect by changing different parameters. Taking this into account, it was considered appropriate to use a slightly different parameter during the optimization in the course of research. In essence, the working culture used during the preparation of planting material was not in a standard nutrient medium, but in a medium prepared on the basis of the substrate on which the fungus spreads. Compared to the planting material obtained from a working culture maintained in an agarized nutrient medium, the use of planting material obtained from a culture maintained in a medium consisting of a substrate in which the fungus lives leads to a relative increase in biomass yield. This fact itself allows us to note that there are still opportunities for optimization. According to all parameters, the amount of biomass obtained during the cultivation of strains selected under optimized conditions for 5 days in BC conditions was 9.7-10.1 g/l.

During the biochemical analysis of the biomass produced by mushrooms belonging to the genus *Ganoderma* P. Karst, including the clarification of the specific gravity of polysaccharides, it became clear that the mushrooms selected as active producers can be considered promising in this regard. Thus, polysaccharides constitute a significant part of the biomass produced by the mushrooms *G. applanatum* S-69 and *G. lucidum* S-76, which were selected as active producers during the research, but the amount of polysaccharides they produce differs from each other (Table 2). As seen, the amount of polysaccharides in the biomass produced by both studied mushrooms is relatively high, but the amount of protein is low. Furthermore, the protein content of *G. applanatum* S-69 is higher than that of *G. lucidum* S-76, but the polysaccharides are the opposite.

The polysaccharides produced by the mushrooms differ from each other, as some of them are soluble, while others are insoluble, and in both mushrooms, the amount of the

insoluble fraction is 11.6-12.0 times greater than the soluble fraction (Table 3). As seen, the fractions also differ from each other in other indicators, which is also reflected in the antimicrobial activity of those fractions (Table 4).

Table 2. Biochemical composition (%) of the biomass of mushroom selected as active producers

| Component composition | <i>G. applanatum</i> S-69 | <i>G. lucidum</i> S-76 |
|------------------------|---------------------------|------------------------|
| Protein | 29,9±0,35 | 28,2±0,21 |
| Reducing sugars (RS) | 54,9±1,57 | 58,0±1,75 |
| Nucleic acid | 0,78±0,02 | 0,73±0,02 |
| Lipids | 3,6±0,10 | 3,8±0,09 |
| Mineral elements (Ash) | 2,4±0,06 | 2,5±0,05 |

Table 3. Chemical analysis of carbohydrate content of mushroom strains selected as active producers

| | SS, % | Protein, % | Ash, % | Monosaccharide composition (%) | | | |
|----------|-------|------------|--------|--------------------------------|---------|-----------|--------|
| | | | | glucose | mannosa | galactose | Othtes |
| S-69(H+) | 6,9 | 1,7 | 0,35 | 62,3 | 18,9 | 10,2 | 8,6 |
| S-69(H-) | 80,3 | 7,1 | 0,76 | 60,1 | 17,8 | 15,2 | 6,9 |
| S-76(H+) | 7,1 | 1,8 | 0,37 | 64,3 | 20,1 | 11,2 | 4,4 |
| S-76(H-) | 85,4 | 7,3 | 0,80 | 61,1 | 18,9 | 16,2 | 3,8 |

Table 4. Bactericidal and fungicidal properties of polysaccharide fractions obtained from active producers (by diameter of lysis zone, mm)

| Test cultures | <i>G. applanatum</i> S-69 | | <i>G. lucidum</i> S-76 | |
|-------------------------------|---------------------------|---------|------------------------|---------|
| | H+ | H- | H+ | H- |
| <i>Staphylococcus aureus</i> | 17±0,50 | 14±0,10 | 21±0,51 | 19±0,63 |
| <i>Bacillus subtilis</i> | 19±0,60 | 16±0,54 | 24±1,00 | 22±0,58 |
| <i>Pseudomonas aeruginosa</i> | 21±1,0 | 17±0,64 | 22±0,44 | 19±0,53 |
| <i>Escherichia coli</i> | 23±0,90 | 19±0,35 | 27±0,47 | 22±1,00 |
| <i>Candida albicans</i> | 16±0,12 | 12±0,05 | 19±0,24 | 17±0,81 |
| <i>Aspergillus flavus</i> | 16±0,35 | 13±0,21 | 18±0,66 | 16±0,34 |
| <i>Mucor hiemalis</i> | 17±0,21 | 14±0,59 | 20±0,68 | 18±0,45 |
| <i>Penicillium cyclopium</i> | 19±0,90 | 17±0,61 | 21±0,86 | 20±0,72 |

As seen, the antimicrobial activity of soluble fractions is higher than that of insoluble fractions, and this is also reflected in antibacterial and antifungal activities. According to the accepted criteria (Bakhshaliyeva et al., 2020), all recorded activities are assessed as weak (less than 20 mm) and moderate (between 20-29 mm). Strong (more than 29 mm) antimicrobial activity is not observed. Despite the above-mentioned differences, the insoluble fraction in both mushrooms is characterized by a high relative amount of 1,3- β -glucosidic bonds (Table 5). In addition, polysaccharide fractions have a higher digestibility compared to total biomass.

All this allows us to note that the studied mushrooms are a promising source as producers of polysaccharides. The main indicators of polysaccharides synthesized by mushrooms (bactericidal and fungicidal activity, lack of toxic effects, high digestibility, relatively high specific gravity of 1,3- β -glucosidic bonds, which are characterized as an indicator of pharmacological activity, etc.) provide a serious basis for their potential as a source of both functionally active food and medically important substances.

Table 5. The ratio of glycosidic bonds in polysaccharide fractions

| | The relative amount of glycosidic bonds,% | | |
|-------------------------------|-------------------------------------------|--------------------------|--------------------------|
| | 1,3- β - glucoside | 1,4- β - glucoside | 1,6- β - glucoside |
| <i>G.applanatum</i> S-69 (H-) | 38 \pm 0,21 | 33 \pm 1,08 | 29 \pm 1,15 |
| <i>G.applanatum</i> S-69 (H+) | 29 \pm 0,24 | 53 \pm 2,10 | 18 \pm 0,24 |
| <i>G.lucidum</i> S-76 (H-) | 41 \pm 1,00 | 32 \pm 1,13 | 27 \pm 0,42 |
| <i>G.lucidum</i> S-76(H+) | 31 \pm 1,04 | 50 \pm 2,21 | 19 \pm 0,47 |

4. Discussion

Xylotrophic macromycetes are characterized as rich sources of bioactive substances, and therefore they are of great practical importance. Thus, many of the metabolites they synthesize have antimicrobial, antioxidant, antiviral, antitumor, etc. activities, and some of them are already used in production conditions. Our studies have shown the widespread distribution of xylotrophic macromycetes in the Republic of Azerbaijan and the presence of mushrooms that are producers of substances with biological, including pharmacological activity, in world practice. For this reason, a collection of 277 strains of mushrooms belonging to 72 species was created. The polysaccharides synthesized by mushrooms belonging to the genus *Ganoderma*, which are currently widely studied as BAS producers in world practice, included in the collection, were evaluated for their biological activity.

Although the results obtained only identify previously recorded species of the studied genus, the creation of a collection of isolated strains is a necessary step for research in this direction. So, for the source of obtaining BAS, are used 2 substances of basidiomycetes: naturally formed FB and pure culture in the vegetative growth phase. The reserves of FB produced by mushrooms, including species of the genus *Ganoderma* P. Karst, in natural conditions are not sufficient (both due to their volume and their lack of production in all seasons of the year), and some species are even listed in the “Red Book” of some countries. True, the solution to this problem has been confirmed using the example of some mushrooms, and a method for intensive cultivation of them has been determined. However, for many xylotrophic macromycetes, including species belonging to the genera *Ganoderma*, *Laetiporus*, *Schizophyllum*, *Trametes*, etc., such a method has not yet found a positive solution. Although mushroom species belonging to these genera have been shown to be suitable as producers of various BAS (Martinez-Burgos *et al.*, 2024, Psurtseva *et al.*, 2023).

When evaluating the biomass yield of 36 strains belonging to 4 species of the genus *Ganoderma* (*G. adpersum*, *G. applanatum*, *G. lucidum*, and *G. resinaceum*), which were evaluated for biomass formation during the vegetative growth phase, it was determined that there were strains with both high, low, and moderate growth rates among them. Compared to the others, the strains with the highest biomass yield were *G. applanatum* S-69 and *G. lucidum* S-76, for which, as a result of optimizing the cultivation environment, biomass yield was achieved up to 9.7-10.1 g/l for 5 days. It is quite difficult to say whether this result is high or low since it is related to the use of different methodological approaches in studies conducted to evaluate mushrooms, including their xylotrophic species, for biomass yield. For example, currently, according to the results of various studies, the biomass yield in strains belonging to xylotrophic macromycetes can be up to 10.5 g/l and 22.23 g/l. However, the amount of nutrients used in these studies is 2-3 times higher than that used in our studies (Bisko *et al.*, 2020), and the methodological approach applied is excessively complex and financially intensive (Romero *et al.*, 2025). According to another study, the biomass produced

by the *G. lucidum* fungus varies between 8.36–10.73 g/l (Liu and Zhang, 2018). In this case, the cultivation period is 7 days, and the composition of the nutrient medium used is more complex. Such cases are common in the literature, which causes difficulties in unambiguously assessing the results obtained.

Among the components of the biomass formed by mushrooms selected as active producers, the amount of total polysaccharides constitutes 54.9–58.0% of the biomass formed by them, more precisely, 5.66–5.74 g/l of the biomass they produce is polysaccharides. This system is of high quality compared to a number of mushrooms studied in world practice. For example, of the 22.23 g/l biomass produced by the mushrooms *Inonotus hispidus*, 8.53 g/l is accounted for by total polysaccharides (Romero *et al.*, 2025). The analogous indicator for the exopolysaccharides of the *G. lucidum* G10016 mushroom is 8.36 g/l and 0.44 g/l (3.68%) (Liu and Zhang, 2018), respectively, which is less than that of the *G. lucidum* S-69 mushroom (7.03%).

As for the chemical composition of the polysaccharides synthesized by the mushrooms, it was clear from the results that the polysaccharides synthesized by the mushrooms *G. applanatum* S-69 and *G. lucidum* S-76 were determined to be composed of glucomannan. This is an expected result, as it is known from many studies (Flores *et al.*, 2024, Wang *et al.*, 2022) that the polysaccharides synthesized by basidiomycetes contain glucomannan.

It should be noted that the glycosidic bonds involved in the formation of the mushrooms' polysaccharides also affect the formation of their biological activity, and in this regard, β -glucans, especially those with a greater participation of 1,3- β -glucoside bonds, are considered more important (Feng *et al.*, 2025). The fact that they are characteristic of polysaccharides obtained from mushrooms, and it is these indicators that provide their promising potential, has been confirmed in a number of studies (Díaz-Montes, 2022, Liang *et al.*, 2024).

5. Conclusion

Most of the natural biopolymers belong to polysaccharides with different chemical and physical properties, and for this reason, they open wide prospects for use in different biological situations. Thus, unlike other synthetic polymers, polysaccharides have a number of properties such as stability, hydrophilicity, biocompatibility, safety, etc. In addition, polysaccharides can be modified chemically and adapted for a specific purpose (for example, the creation of pharmacoseptic materials, plasma substitutes, etc.). All this leads to increased interest in them, especially in the medical field, and an increase in the number of studies devoted to the search for their active producers.

Among the biologically active substances synthesized by mushrooms are polysaccharides, which can be synthesized by all taxonomic structures of mushrooms: ascomycetes, basidiomycetes, and even fungus-like organisms. The polysaccharides they synthesize differ from each other in composition and structure; therefore, to obtain the maximum amount of fungal polysaccharides, the process (selection of the fungal strain, evaluation, inoculation, incubation, isolation, and purification of the target product) must be optimized for each species and even strain as a whole.

It is true that recent research in this field has yielded interesting results from both a scientific and practical perspective, and significant progress has been made in the structural properties, biological activities, and applications of polysaccharides in biotechnology. However, there are still some gaps in both the fundamental and applied aspects of this issue, which is primarily due to the extreme diversity of biological properties and pharmacological activities of fungal polysaccharides. This, in turn, leads to the expansion of research on mushrooms in this aspect and creates confidence in the promising use of mushroom polysaccharides in agriculture, medicine, the food industry, the preparation of cosmetics, etc. Although mushroom polysaccharides have great potential for application in various fields, their practical application

is not widely found and is mainly based on experimental results. There are a number of reasons for this, primarily due to the fact that the methodological approaches used to obtain mushroom polysaccharides are not very favorable due to economic and technological considerations, and the number of cultures studied for this purpose covers an extremely small part of the mushrooms, etc. Therefore, research in this direction should primarily focus on finding new crop producers from nature, expanding production processes, reducing costs, expanding the application of integrative methods, and other issues.

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Conflict of interests

The authors declare they have no conflict of interest to declare.

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