

Obtaining active polysaccharides from basidiomycete fungal strains

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ARTICLE INFO	ABSTRACT
Article history: Received 01.12.2025 Received in revised form 03.02.2026 Accepted 26.02.2026	After extraction of polysaccharides from various strains of basidiomycetes grown in nature and on various nutrient media, the physico-chemical properties of the obtained samples were studied using several spectral methods. The purity of polysaccharide fractions was determined for the first time. It was found that the isolated polysaccharides are branched beta-glucan type polysaccharides with branching points.
Keywords bioactivity, cultivation, biomass, fungal strains, polysaccharides	

Basidiomycetes can serve as sources of biologically active metabolites that are promising for use in biopharmaceuticals and biomedicine. Particular attention is paid to the development of methods for isolating biologically active substances from basidiomycetes, which are a promising object of research - determining the structure and bioactivity of the resulting bioactive compounds, as well as developing pharmaceuticals based on them that have immunomodulatory and antitumor properties [1,2]. In recent years, basidiomycetes have become the subject of in-depth research in terms of searching for new antitumor and various bioactive substances. Researchers have become increasingly interested in studying the cell wall composition of basidiomycetes. The polysaccharides they contain are among the most important components of organic compounds and are involved in many important biological processes. The polysaccharides contained in basidiomycetes accumulate in fruiting bodies and spores, and their concentration is particularly high in the mycelium [3]. These polysaccharides, as mentioned above, vary in their chemical structure, but all contain β -(1.3)-glucan groups in the main chain and β -(1.6)-glucan groups in the branched regions, which are responsible for biological activity. Chromatography-mass spectrometry is often used for the structural analysis of polysaccharides. The extracted polysaccharides are light brown powders, well soluble in water, forming an opaque solution at low concentrations and a viscous solution at high concentrations [4]. This method allows one to obtain information on the retention times of its components, their relative abundance in the mixture, and the mass spectra of each component in a single analysis. As natural raw materials for the extraction of water-soluble polysaccharides, selected basidiomycetes *Ganoderma lucidum*, collected from natural habitats in Republic of Azerbaijan, as well as those obtained by cultivation. Currently, scientific research is underway in Republic of Azerbaijan to identify natural sources rich in biologically active compounds, to isolate them, and to determine their structure and biological activity [5,6,7].

Methods

Cultivation process. From pure cultures of the fruiting bodies of this mushroom, a local strain, *Ganoderma lucidum*, was obtained for the first time using cultivation. To isolate water-soluble polysaccharides from the natural raw material, dried, crushed, and defatted tinder bodies were used, weighed, and extracted three times with hot water in a boiling water bath with a reflux condenser (Fig. 1 and 2). A water-soluble polysaccharide sample (100 mg) was dissolved in 5 ml of distilled water and loaded onto a column (14 × 3 cm). The polysaccharides were eluted sequentially

with a 0–1 M NaCl gradient solution at a flow rate of 60 ml/h. Fractions of 10 ml were collected. The polysaccharide yield from the column was monitored using the phenol-sulfuric acid method (Soy flour + glucose; Peptone + glucose; Yeast extract + sodium nitrate + glucose; Soy flour + vegetable oil; Peptone + vegetable oil; Yeast extract + sodium nitrate + vegetable oil). The fractions were pooled, concentrated, dialyzed, and lyophilized (Fig.4). A 5 mg sample of the polysaccharide was dissolved in 1.5 ml of dimethyl sulfoxide, 30–40 mg of finely ground sodium hydroxide was added, and then 0.5 ml of CH_3I was added with stirring. The reaction mixture was left for 1 hour, 5 ml of chloroform was added, and the mixture was washed several times with water. The chloroform solution was then evaporated, and the residue was hydrolyzed by heating with 1 ml of 2 M trifluoroacetic acid at 100°C for 8–9 hours. The acid was removed by evaporation with ethanol, and the resulting methylated monosaccharide derivatives were converted to polyol acetates and identified using a known method using chromatograph mass spectrometry. The cultivation process involved two stages: growing liquid seed material and accumulating the fungal biomass (Fig. 3).

Results. As a result of the study, branched polysaccharides were isolated from basidiomycete raw materials (locally grown and cultivated). The isolated fractions contain branched polysaccharides in the form of complexes with melanin.

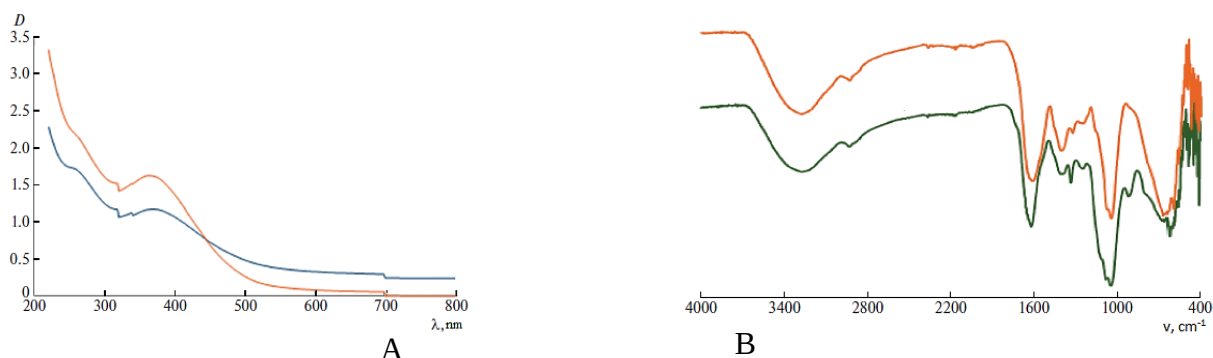
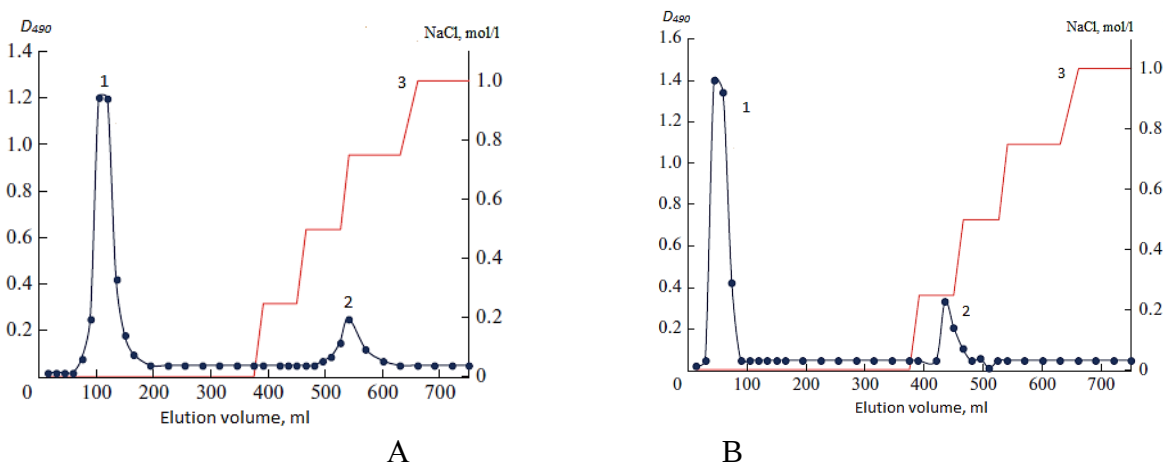


Figure 1. A - Spectrum of water-soluble polysaccharide fractions isolated from locally grown GW (red line) and cultivated GWL (blue line) basidiomycete raw materials; B - Infrared spectroscopy of isolated aqueous fractions of GW (orange line) and GWL (green line) polysaccharides



Note: 1 - neutral polysaccharides from locally grown basidiomycete raw materials; 2 – anionic polysaccharides; 3 - Eluent is a gradient solution of NaCl 0 - 1.0 mol/l, the elution rate is 1 ml/min.

Figure 2. A - Ion-exchange chromatogram of polysaccharides GW (1) and GW (2) in a DEAE-52 cellulose column; B - Ion exchange chromatogram of polysaccharides GWL (1), GWL (2) in a DEAE-50 cellulose column.

The degree of branching of β -D-glucans for locally growing basidiomycetes (GW) was proven to be 0.74, while for cultivated basidiomycetes (GWL) it was 0.38. Submerged cultivation allows for the production of biomass containing biologically active metabolites. This study demonstrated interferon-inducing activity of the total fraction of water-soluble polysaccharides, which is comparable to that of the positive control.

Table 1.*Amount and molecular weight of carbohydrates after fractionation*

Basidiomycetes	Solvent	Polysaccharide	Amount of polysaccharide, %	Impurities, %
Locally growing	Water	GW-1	98.21	0.61
	0.1 M NaCl	GW-2	64.01	69.78
Cultured	Water	GWL-1	97.32	0.70
	0.1 M NaCl	GWL-2	77.54	66.50
Molecular weight parameters of polysaccharides				
Basidiomycetes	Fraction		$M \times 10^{-3}$	M_w/M_n
Locally growing	Water Fraction (GW)		12.2	1.5
	Neutral Polysaccharide (GW-1)		17.5	1.4
	Anionic Polysaccharide (GW-2)		10.4	1.4
Cultured	Water Fraction (GWL)		10.8	1.4
	Neutral Polysaccharide (GWL-1)		10.3	1.4
	Anionic Polysaccharide (GWL-2)		9.7	1.2

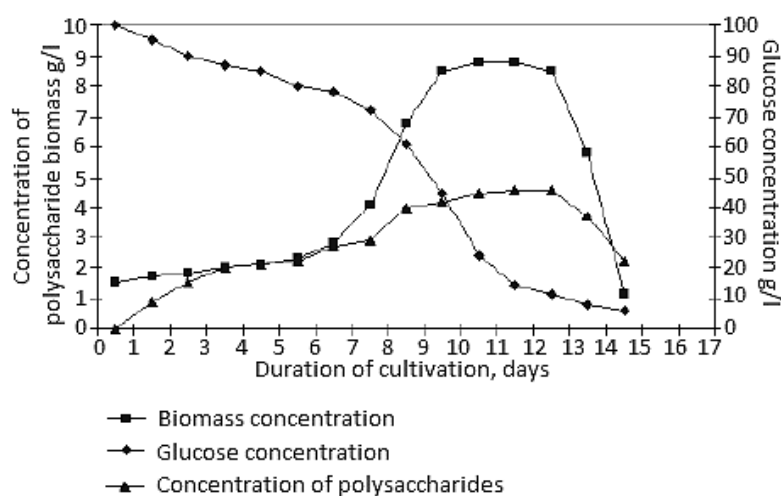
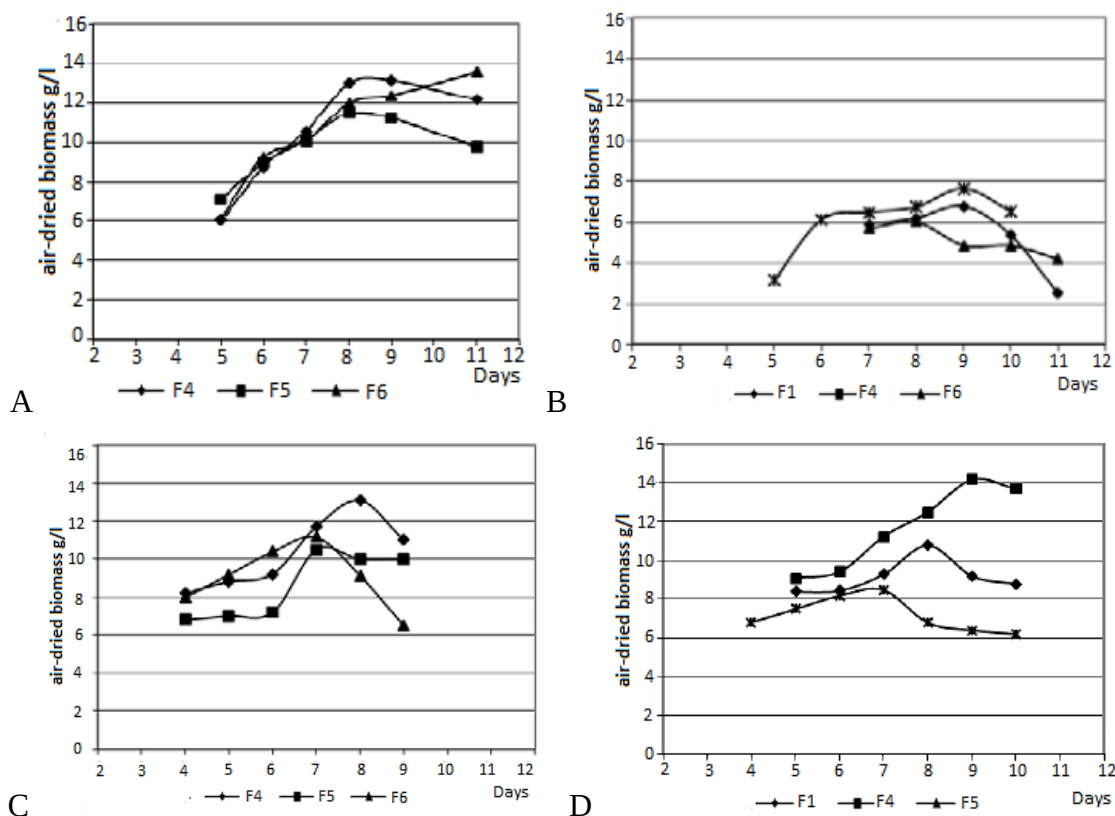


Figure 3. Dynamics of glucose consumption, accumulation of fungal biomass and polysaccharides (Cultivation in pure and together forms with each of the strains to be screened, in a liquid medium).

As a result of preliminary studies, three environments were selected for each strain that provided the highest yields of their biomass (Fig. 4). The process of submerged cultivation was studied on the media selected for each strain. The duration of experiments to study the dynamics of biomass accumulation of all strains did not exceed 12 days, regardless of whether the culture reached the last phase stage or not (Fig. 4 A-D).



Note: F1 - Soy flour + glucose; F2 - Peptone + glucose; F3 - Yeast extract + sodium nitrate + glucose; F4 - Soy flour + vegetable oil; F5 - Peptone + vegetable oil; F6 - Yeast extract + sodium nitrate + vegetable oil

Figure 4. Accumulation of biomass (Main results) of the studied fungal strains on selected media

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Xülasə

Basidiomiset göbələyinin şammlarından fəal polisaxaridlərin alınması

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Açar sözlər: *bioaktivlik, əkilmə, biokütlə, göbələk şammları, polisaxaridlər*

Təbii halda və müxtəlif qidalı mühitlərdə yetişdirilən basidili göbələklərin müxtəlif şammlarından polisaxaridlərin alınmasından sonra, əldə edilən nümunələrin fiziki-kimyəvi xüsusiyyətləri bir neçə spektral metodlardan istifadə etməklə öyrənilmişdir. İlk dəfə olaraq, polisaxarid fraksiyalarının saflığı müəyyən edilmişdir. İzolyasiya edilmiş polisaxaridlər şaxələnmə nöqtələrinə malik şaxələnməmiş β -qlükan tipli polisaxaridlər olduğu müəyyənləşdirilmişdir.

Резюме

Получение активных полисахаридов из штаммов базидиомицетных гриба

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Ключевые слова: *биоактивность, культивирование, биомасса, штаммы грибов, полисахариды*

После экстракции полисахаридов из различных штаммов базидиомицетов, выращенных в природе и на различных питательных средах, физико-химические свойства полученных образцов были изучены с использованием нескольких спектральных методов. Впервые была определена чистота полисахаридных фракций. Было установлено, что выделенные полисахариды представляют собой разветвленные полисахариды β -глюканового типа с точками разветвления.