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# ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

# BIOTECHNOLOGICAL POTENTIAL OF MUSHROOMS BELONGING TO THE GENUS GANODERMA SPREAD IN THE FOREST ECO-SYSTEMS OF AZERBAIJAN AS A PRODUCER OF POLYSACHARIDES

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# INTRODUCTION

**Relevance and development of the topic.** It is known, that fungi, especially their xylotrophic species, play an important role in ensuring their sustainability by actively participating in the destruction and transformation of organic matter in forest ecosystems. In addition, they are of economic importance as reclamation, medicinal and food resources. Azerbaijan is included in the list of low-forested countries, and "*today 10.4% of its territory is covered by forests*"[1], and one of the most studied and found places for fungi is forests. The mycopotential of this or that forest, especially those located in the territory of the Republic of Azerbaijan, can be estimated relatively. This is due to the fact, that in the last 20 years, there has been a lack of research on forests in the territory of the Republic of Azerbaijan, and much of the research is dedicated to local mycobiota. On the other hand, the work carried out both in Azerbaijan and in other countries of the world is mainly mycocenotic. Thus, the research identified the species composition, phenological characteristics, ecology and trophic groupings of fungi found in the selected route or permanent area. The work carried out on the basis of this methodological approach is aimed at solving the main problem of mycosenology, ie the true boundaries of the grouping of macromycetes, their potential as a bioresource, etc. does not allow determining the characteristics.

However, recent interest in fungi is not only focused on the problems of mycocenology, but also as a promising source of biologically active substances (BAS) with healing properties for some

[1] Məmmədov Q., Xəlilov M. Azərbaycan meşələri.Bakı:Elm, 2002, 472s.

time, and this attention is growing. According to the results of research conducted in recent decades, "*fungi are rich in proteins, polysaccharides, lipids, organic acids, enzymes, vitamins and others there is no doubt, that BAS is a producer*"[2,3,4.] Many of these types of BAS have pharmacological activity and are less toxic and more effective when used in medical practice than those obtained by chemical synthesis. Due to these qualities, the formula of many biologically active additives based on the fruit bodies of higher basidiomycete's mushroom has been patented.

In these studies, the substance used to obtain BAS was preferred to use the fruit body formed by fungi grown both naturally and artificially, although the vegetative mycelium of macromycetes can also be used as a source for the production of BAS and their resources to FB. can be obtained at any time and at any time of the year. Despite these advantages, the number of xylotrophic macromycetes studied in this area is extremely small, and many species have not been studied at all in this regard. If we add to the above that the quantitative indicators of the synthesis of this or that BAS are formed depending on the substrate itself and the natural environmental conditions of the area where the fungus is isolated, then there is no doubt about the application of individual approaches to this or that species.

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[2] Atri, N.S., Guleria L. Evaluation of vitamin, phytohormone and trace element requirements of Lentinus cladopus Lév// International Journal of Pharmacy and Pharmaceutical Sciences. – 2013. – Vol. 5 (4). – P. 40–42.

[3] Wasser S.P. Medicinal mushroom science: Current perspectives, advances, evidences, and challenges.// Biomed. J. 2014, v.37, p.345–356

[4] Xu X., Yan H., Chen J., Zhang X. Bioactive proteins from mushrooms // Biotechnol Adv., 2011, v.29, № 6, p.667-674.

As noted, one of the BAS spectra of fungi is "*polysaccharides*"[5], which are also currently used for various purposes (medical, feed and food) as compounds with different functional activities. The fact, that the biological activity of not all of the used producers does not meet the required requirements is one of the most important areas of research.

**Aim and tasks.** The purpose of the present work is to study the physiological-biochemical and biotechnological bases of the use of fungal species of Ganoderma P.Karst as a producer of polysaccharides, which are widespread in Azerbaijan.

It is planned to solve the following tasks to achieve the set goal:

- Separation and creation of a collection of pure cultures of Ganoderma mushroom distributed in different areas from the ecological point of view of the Republic of Azerbaijan;
- Evaluation of fungal strains of the genus Ganoderma according to cultural-morphological and growth indicators in different conditions;
- Comparative assessment of the biochemical composition of vegetative mycelium and fruit bodies of fungal species belonging to the genus Ganoderma;
- Separation of polysaccharide fraction of fungal species belonging to the genus Ganoderma and study of its bactericidal and fungicidal properties.

**Research methods.** In dissertation mycological as well as biochemical methods and approaches currently used in similar works were used in the research envisaged. It was carried out according to the

[5] Ruthes A.C., Smiderle F.R., Iacomini M. Mushroom heteropolysaccharides: A review on their sources, structure and biological effects.// Carbohydr. Polym., 2016, v.136, p.358–375.

route method for collecting the fruit body of the fungus selected for research. Obtaining a pure culture of mushrooms, extracts from the fruit body and vegetative mycelium of mushrooms using water and alcohol, their antimicrobial activity, toxicity, biochemical composition, etc. Methods and approaches that are currently widely used in research in this area have been used to study the characteristics of It was considered expedient to have at least 4 repetitions of the experiments, to allow statistical processing of the obtained results, to use in the dissertation only the data that do not doubt the accuracy, meet the formula m / M = P≤0.05.

**The main provisions of the dissertation submitted for defense:**

• Although there are 4 species of *Ganoderma* in ecologically different areas of Azerbaijan, such species as *G.lipsiense* and *G.lucidum* are characterized by higher incidence in Azerbaijan;

• Although Ganoderma fungi differ in the amount of biomass they produce, there are many strains with high growth rates;

• Although the biomass produced by fungi of the genus Ganoderma, as well as polysaccharides, differ somewhat in quantity, their main constituent element is β-glucans:

• The absence of toxic substances in the biomass formed by fungi of the genus Ganoderma, rich in biologically active metabolites, are serious grounds for confirming that their use in food, feed and medical purposes is more promising.

**Scientific novelty of the research.** As a result of research carried out in the territories of Azerbaijan differing in natural soil-climatic conditions, the distribution of Ganoderma species such as *G.adspersum, G.lipsiense* (= *G.applanatum*), *G.lucidum* and *G.rezinaseum* was determined and their separate territorial units and substrates The distribution, the lifespan of the fruit body (FB) formed under natural conditions, ecological and trophic relationships, the color of decay caused by natural conditions, the synthesis of metabolites with biological activity, as well as the possibilities of their use were clarified.

It has been shown, that the growth rate of strains of Ganoderma fungi from perennial fruit-bearing species is higher than that of strains from relatively young fruit bodies, and 27.1% of the collection of 59 strains of Ganoderma species is fast, 35.6%. medium and 37.3% have late growth capacity.

*G.lipsiense* S-17 and *G.lucidum* S-42, which are classified as active strains according to their growth rate, should be grown in a liquid glucose-peptone medium to maximize the biomass formation of the fungi, as well as the amount of glucose added to the environment. -9.7 g/l, peptone 3.0 g/l, $NH_4NO_3$ - 1.5 (0.037-0.038% for nitrogen), cultivation temperature $28^0$C, initial acidity of the environment 5.5, cultivation lasts for 5 days It has also been experimentally determined, that it is more convenient to do.

It has been found that although glucose is the optimal carbon source for the active producers of *Ganoderma* mushroom to produce maximum biomass, changing the carbon source causes a change in the proportions of the polysaccharides synthesized.

Parts of strains selected as high-growth producers extracted with water and alcohol from both FB and VM, which are formed naturally, have biological activity. In this case, the biological activity in *G.lucidum* S-42 strain is always increased, and in *G.lipsiense* S-17 the increase and decrease observed compared to the initial sample is not significant and in all cases is less than in the control variant. A similar situation is observed in metabolites secreted by the latter strain into the culture solution.

It was found that 67.4% of the biomass formed by the fungus *G.lucidum* S-42 is polysaccharides, 1/12 of which consists of soluble and the rest insoluble fractions, and both fractions have bactericidal and fungicidal activity. In addition, it is characterized by a high degree of digestibility relative to biomass and a large amount of 1,3-β-glucoside bonds, which are considered pharmacologically valuable.

**Theoretical and practical significance of the research**. The results obtained are factual material that serves to expand the understanding of the potential of xylotrophic macromycetes as fungi of the genus *Ganoderma* as producers of polysaccharides.

*Ganoderma* fungi, especially *G.lucidum*, selected as a polysaccharide producer of xylotrophic macromycetes, are a reliable source of various products and can be successfully used to solve the problem of food shortages in the world.

The biochemical parameters of the obtained polysaccharide fractions, first of all, their non-toxic effect, high digestibility, relative relative content of glucoside bonds are serious grounds for their perspective as an important source in the purchase of feed and food, as well as medical products.

**Publication, approbation and application of the dissertation.** 16 works related to the dissertation were published, 12 of them are the scientific articles.

Materials of dissertation were presented at the Republican Scientific Conference on "Actual Problems of Soil Science" (Baku, 2017), at the VII Republican Scientific Conference on "Actual Problems of Ecology and Soil Science in the XXI Century" (Baku, 2018), at the International Conference on "Development of Science in the XXI Century"(Kharkiv, 2018) and at the scientific conference on "Actual problems of modern biology"(Baku, 2019).

**Organization, where the dissertation work is performed.** The dissertation was accomplished in the laboratories of microbiological biotechnology and biologically of the Institute of Microbiology of Azerbaijan National Academy of Sciences.

**Structure and scope of the dissertation**. The dissertation consists of an introduction and 4 chapters, a final analysis of the results, the main results, a list of references, appendices and a list of abbreviations used in the dissertation. The dissertation consists of 137 pages, including tables and figures, as well as a list of references, which makes a total of 219210 characters.

## LITERATURE REVIEW
## CHAPTER I
## GENERAL CHARACTERISTICS OF BASIDIL MUSHROOMS AS PRODUCERS OF BIOLOGICAL ACTIVE SUBSTANCES

Part 1.1 of the dissertation provides information on fungi that make up a group of existing biodiversity on earth and their ecological functions in nature, part 1.2 provides information on the fact that mushrooms are a promising object of biotechnology, and part 1.3 provides information on Ganoderma P. Carst fungi in the world and Azerbaijan. Results of researches devoted to the study of widespread species and their role as producers of BAS are analyzed and their level of study is assessed.

## MATERIAL AND METHODOLOGY OF THE WORK
## CHAPTER II

### 2.1.-2.2. Research areas and methods used for analysis

The research was conducted in natural and artificial forests in the Greater Caucasus(GC), Lesser Caucasus(LC), Kur-Araz lowland(KA) and Lankaran-Astara(LA) zone of Azerbaijan.

The fruit body of the genus Ganoderma of xylotrophic macromycetes distributed in the forests of the selected sites was collected according to known "*methods*"[6] (route and permanent site selection), certified on site and prepared for the laboratory analysis. In the laboratory, the FB was identified as a species based on known "*determinants*"[7] and pure culture was isolated. Bleached malt juice (ASH, 2-30B) was used to obtain pure culture. The purity of the culture is monitored using a microscope (OMAX 40X-2500X LED Digital Lab Trinocular Compound Microscope). For the production of vegetative mycelium from fungi, a liquid glucose-peptone medium (LGPM) was used, the composition of which was as follows (g / l): glucose -10, peptone -3, $NH_4NO_3$ - 1.5, NaCl - 0.5, $MgSO_4 x 7H_2O$ - 0.5, $K_2HPO_4$ - 0.4 and $FeSO_4$ - traces.

For the production of biologically active substances (BAS), a biomass consisting of the naturally formed fruit body (FB) of Ganoderma fungi and the vegetative mycelial (VM) formed during the cultivation of the pure culture in LGPM, as well as in the latter condition cultural solution (CS) were also used.

During the cultivation of mushrooms in pure culture, they were also studied according to the growth factor (GR), which was also used in the calculation of the "*method*"[8] used in the work of A.S. Buhalo.

To separate the exopolysaccharides from CS, the solution obtained after filtration is thickened several times using a vacuum rotor evaporator. The solid solution is treated with 96% ethyl alcohol in a

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[6] Foster M., Mueller G., Bills G. Biodiversity of fungi. Inventory and monitoring methods. Boston. Elsevier Academic Press. 2004. 777 p.

[7] Бондарцева М.А. Определитель грибов России. Порядок афиллофоровые. СП.:Наука, 1998, вып. 2, 391с.

[8] Бухало А.С. Высшие съедобные базидиомицеты в чистой культуре. Киев: Наукова думка, 1988, 176 с.

ratio of 1: 2, and the precipitate obtained (by centrifugation for 15 min, 5000 rpm) is dried and powdered and analyzed as an exopolysaccharide fraction.

Separation of the polysaccharide fraction from the mycelium was carried out according "*to the method*"[9,10]used in the work of various authors.

In determining the qualitative composition of polysaccharide fractions obtained from fungi, a "*method*"[11] based on the complete hydrolysis process with the help of acids was used.

Quantitative analysis of polysaccharide fractions obtained from fungi was carried out on the basis of gas-liquid chromatography and protein content by "*Loru methods*"[12].

Antimicrobial activity of polysaccharide fractions obtained from the studied mushroom was carried out according to "*known methods*"[13] using traditional test cultures.

In the course of the research, all experiments were performed in 4-6 repetitions, all the results were "*statistically processed*"[14] and in all

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[9] Захарова И.Л., Косенко Л.В. Методы изучения микробных полисахаридов. Киев: Наукова думка, 1982, 189 с.

[10] Liang Z., Yi Y., Guo Y. et. al Chemical characterization and antitumor activities of polysaccharide extracted from *Ganoderma lucidum* // Int. J. Mol. Sci., 2014, v. 15, № 5, p. 9103-9116.

[11] Оленников, Д. Н., Рохин А. В. Водорастворимые глюканы семян кардамона настоящего Elettaria cardamomum // Приклад. биохимия и микробиол. – 2013. – т. 49, № 2. – с. 197–202.

[12] Практикум по биохимии (Под. ред. Н.П.Мешковой и С.Е.Северина.). М: МГУ, 1979, 430 с.

[13] Егорова Н. С. Руководство к практическим занятиям по микробиологии. Учеб. Пособие. 3-е изд., перераб. и доп.М.: Изд-во МГУ, 1995, 224 с:

[14] Гланц, С. Медико-биологическая статистика. М.: Практика, 1998. – 459 с.

cases the results correspond to the formula m / M = P≤0.05 (M is the average value of repetitions, m is the standard deviation, P is the Student's criterion) was used.

# EXPERIMENTAL PART
# CHAPTER III
# DISTRIBUTION OF GANODERMA MUSHROOMS IN AZERBAIJAN AND EVALUATED BY THEIR GROWTH ABILITY, CREATION OF THE COLLECTION

## 3.1. Species of Ganoderma mushroom, distributed in ecologically different areas of Azerbaijan

In order to create the collection of a pure cultures of Ganoderma fungi, 109 FBs, which are believed to be xylotrophic macromycetes, primarily Ganoderma mushroom, were collected from trees in natural and artificial forests in the study areas, and laboratory studies revealed that 75 of them were Ganoderma species was determined to belong. 59 pure cultures were allocated from this FB, their species composition and distribution of Azerbaijan by geomorphological units are summarized in table 3.1.

**Table 3.1**

**Distribution of strains of the genus Ganoderma by individual geomorphological units and species**

| | Species | GC | LC | LA | KA | Total |
|---|---|---|---|---|---|---|
| 1 | *G.adspersum* | 3 | 2 | 4 | 1 | 10 |
| 2 | *G.lipsiense* | 8 | 4 | 6 | 2 | 20 |
| 3 | *G.lucidum* | 7 | 5 | 3 | 2 | 17 |
| 4 | *G.rezinaseum* | 3 | 3 | 5 | 1 | 12 |
| Total | | 21 | 14 | 18 | 6 | 59 |

It should be noted that the lifespan of the FB used to separate the strains is also different. Thus, the FB formed by G. lipsiense under natural conditions is plural, and the life expectancy of FB belonging to G. lucidum is unitary. Therefore, all FBs used for the second type were 1-year. Due to the FB multiplicity of G. lipsiense, those with different lifespans were also used to obtain pure cultures. Of particular interest is whether the survival of the FB used to obtain a pure culture affects the growth of the strains derived from it. For this reason, it was considered expedient to clarify this issue in the course of the research. In this regard, it became clear that the survival of FB was determined to affect the growth rate of the strains isolated from them (Table 3.2). As can be seen, the increase in the age of the FB is reflected in the increase in the growth of the isolated strains. For this reason, strains isolated from older FBs were used in subsequent studies. For this reason, only 7 of the 20 strains of G. lipsiense were used in the later stages of the study.

**Table 3.2**

**Characteristics of G.lipsiense strains derived from fruit bodies of different ages, according to the growth rate**

| Age of FB | Growth rate, mm / day | Growth coefficient |
|---|---|---|
| 1 | 0,92 | 28,7 |
| 2 | 0,96 | 32,5 |
| 3 | 1,02 | 38,8 |

It was not possible to determine the presence of a similar situation in the fungi G.adspersum and G.rezinaseum. Thus, these fungi are not considered to be the most widespread species of fungi in Azerbaijan, and therefore, in the course of research, only 22 FB belonging to these two species were found, of which 2 were binary and 20 were 3-years.

For this reason, strains isolated from the 3-year MC were used during the initial screening of fungal strains for biomass yield.

The next stage of the research was the quantitative screening of biomass of fungal species isolated from pure culture. It became clear from the results that although all the strains tested had the ability to grow in Liquid Glucose Peptone Environment used for a cultivation, also they differed in the quantitative value of the biomass they formed (Table 3.3). As can be seen, difference between the maximum and minimum amounts of biomass produced by the tested strains is 6.8 times. This is 5.8 times that of G.resinaseum. Despite these differences, based on the results obtained

**Table 3.3**

**Characterization of Ganoderma strains by amount of the produced biomass (7 days, DC)**

| № | Species | Number of the strains | User strain and amount of the biomass, g / l | | |
|---|---|---|---|---|---|
| | | | Fast-growing | Late-growing | Medium-growing |
| 1 | *G.adspersum* | 9 | 1/ 5,4 | 5/1,2-2,7 | 3/3,2-4,6 |
| 2 | *G.lipsiense* | 13 | 7/5,8-7,5 | 6/1,5-2,9 | 7/3,1-4,8 |
| 3 | *G.lucidum* | 17 | 6/5,2-7,2 | 6/1,3-2,1 | 5/3,6-4,5 |
| 4 | *G.rezinaseum* | 11 | 2/5,1-6,4 | 4/1,1-2,8 | 5/3,3-4,6 |

should be noted, that the tested strains are fast-growing (with a biomass of more than 5 g/l for 7 days), medium (3-5 g/l for 7 days) and weak (less than 3 g/l for 7 days). It can be divided into 3 parts: *G.lipsiense* S-17 and *G.lucidum* S-42 were selected as active producers as a result of this stage.

### 3.2. Optimization of the environment for the process of biomass release of strains selected as an active producer

The Liquid Glucose Peptone Environment, which is grown for maximum biomass formation in strains selected as the active producer, is optimized for key parameters. The main parameters specified for optimization were carbon and nitrogen source, cultivation temperature and initial pH of the environment, method and duration of preparation of planting material (inoculum), as well as cultivation period. Results showed, that the glucose for both fungi as a carbon source, $NH_4NO_3$ for the mineral nitrogen source, the cultivation temperature $28^0C$, pH = 5.5-5.7, the biomass of fungi such as planting material obtained under deep cultivation in liquid glucose peptone medium is suitable and under these conditions the maximum biomass can be obtained as a result of cultivation in both fungi for 5 days.

### 3.3. An annotated list of species of fungi belonging to the genus Ganoderma T. Karst, distributed in Azerbaijan

It was also considered the appropriate to compile an annotated list of fungal species whose distribution was noted in a course of the research, but it was not carried out in accordance with the classical approaches used by most researchers during its compilation. More precisely, those that differed from the classical approach (variation of FB and VM depending on environmental conditions, growth rate, and etc.) have been used too.

# CHAPTER IV

# PHYSOLOGICAL-BIOCHEMICAL AND BIOTECHNOLOGICAL BASIS OF THE POTENTIAL OF POLYSACHARIDES OF MUSHROOMS OF GANODERMA

## 4.1. Determination of toxicity of mushroom selected as an active producer

Primary requirement for food and medical products, as well as feed products, is that they do not have toxic effects. From the results of research to determine this, it became clear that the strains selected as the active producer differ from each other in this respect, and there are some differences depending on the solutions used for the extraction (table. 4.1). As can be seen, none of the materials derived from both the fruiting body and the vegetative mycelium belonging to the *G.lucidum* S-42 strain are toxic, and even stimulation of the growth of the infusor used for toxicity is observed. It also allows to note the presence of metabolites with biological activity in all 3 instances. The results are slightly different in the *G.lipsiense* S-17 strain, as no toxicity is observed in water-extracted and CS, but no significant stimulation is observed. This situation is also observed in the material received from both instances. In an extractable solution with alcohol toxicity is observed, albeit to a lesser extent, and is expressed in relatively high quantities when taken from the fruit body. All this allows us to note that the metabolites formed by the two species have different effects, as well as their solubility in alcohol and water.

It is clear from Table 4.1 that the same materials obtained from both substances show similar results in terms of toxicity. Therefore,

**Table 4.1**

**Toxic activity of strains, selected as active producers in relation to Tetrahymena pyriformis**

| | | Substance | Number of primary cells (cell/300 mkl) | Number of cells after 24 hours (cell/mkl) | Growth factor |
|---|---|---|---|---|---|
| Water | | | | | |
| 1 | *G.lucidum* S-42 | VM | 167 | 489 | 2,92 |
| | | FB | 165 | 474 | 2,87 |
| 2 | *G.lipsiense* S-17 | VM | 155 | 170 | 1,09 |
| | | FB | 162 | 170 | 1,04 |
| 3 | Water | | 145 | 180 | 1,24 |
| Alcohol (1%) | | | | | |
| 1 | *G.lucidum* S-42 | VM | 164 | 428 | 2,60 |
| | | FB | 159 | 401 | 2,52 |
| 2 | *G.lipsiense* S-17 | VM | 144 | 139 | -1,03 |
| | | FB | 150 | 132 | -1,13 |
| 3 | Alcohol | | 138 | 195 | 1,41 |
| CS | | | | | |
| 1 | *G.lucidum* S-42 | | 164 | 320 | 1,95 |
| 2 | *G.lipsiense* S-17 | | 160 | 184 | 1,15 |
| 4 | LGPM | | 147 | 218 | 1,48 |

given the adequate supply of vegetative myceliums and the possibility of obtaining those at any time of the year, as well as the fact that many of the substrates used to produce VM constitute wastes considered sources of environmental pollution expedient to use.

## 4.2. Biochemical contents of vegetative myceliums and culture solutions

It should be noted that, the quality of both FB and VM produced by mushroom is determined by protein and amino acid composition, carbohydrates, lipids, organic acids, vitamins, as well as minerals and extractable substances. For this reason, it was considered expedient to carry out biochemical analysis of VM and CS obtained from active producers in the research. It became clear from the results that polysaccharides make up a significant part of the produced biomass (VM). (table 4.2).

As can be seen, the strains selected as the active producer due to the large component composition do not differ significantly from each

**Table 4.2.**

**Biochemical contents of biomass of the strains, selected as active producers**

| Ingredients | G.lipsiense S-17 | G.lucidum S-42 |
|---|---|---|
| Protein | 22,2 | 21,6 |
| Reduced sugars (RS) | 66,4 | 67,4 |
| Nucleic acid | 0,75 | 0,71 |
| Lipids | 2,6 | 2,9 |
| Mineral elements | 1,4 | 1,5 |

other and observed differences are mainly quantitative. However, the main part of the biomass formed by both fungal cultures is sugars, ie polysaccharides, which also consist of two fractions. The first fraction is soluble (H +) and the second is insoluble (H-). The H + / H- ratio is 1/12 in both fungi. As a result of chemical analysis of both fractions, it became clear that they contain a large number of glucomanes, which is found in both soluble and insoluble fractions (Table 4.3). This presence makes polysaccharides of both fungal strains of practical importance.

**Table 4.3**

**Chemical analysis of the carbohydrate contents of mushroom strains, selected as active producers**

| | Soluble sugars % | Protein, % | Ash, % | Monosaccharide composition (%) | | | |
|---|---|---|---|---|---|---|---|
| | | | | Glucose | Mannoza | Galactose | Others |
| S-17(H+) | 6,7 | 1,7 | 0,35 | 62,3 | 18,9 | 10,2 | 8,6 |
| S-17(H-) | 80,4 | 7,1 | 0,76 | 60,1 | 17,8 | 15,2 | 6,9 |
| S-42(H+) | 7,1 | 1,8 | 0,37 | 64,3 | 20,1 | 11,2 | 4,4 |
| S-42(H-) | 85,2 | 7,3 | 0,80 | 61,1 | 18,9 | 16,2 | 3,8 |

As a rule, the application of these compounds is aimed at either the medical or food industries, at least because the biomass inherent in both fungi, including polysaccharide fractions, is not toxic, but this is the final decision on their application. not enough to give. The issues clarified in the research conducted in this direction are the antimicrobial activity of these fractions, the amount of glucoside bonds in them, various spectral clinical studies, etc. reflects.

With this in mind, studies were carried out to determine the antimicrobial activity, more precisely, bactericidal and fungicidal properties of polysaccharide fractions obtained in the next stage of research, as well as the ratio of glucoside bonds in these fractions.

It was considered expedient to characterize the polysaccharides synthesized by *G.lucidum* fungus selected as an active producer in the course of research using the method of high-efficiency liquid chromatography (HELCH). From the results of the analysis of the culture solution of the fungus *G.lucidum,* it became clear that the analyzed fractions are a mixture of two different types of compounds.

In general, 4 of the 5 recorded fractions consist of polysaccharides that are well soluble in water as a result of physicochemical analysis (Table 4.4).

The constituent elements of these polysaccharides are auxochromic hydroxyl groups, which are not absorbed by ultraviolet (UV) rays. Polyacetylenes (PA) and polysaccharides account for 70-80% of the biomass extracts of mushrooms from ether and water.

Study of molecular-weight properties of individual fractions by the HELCH method allowed to determine the ratio of individual fractions, as well as to determine the optimal conditions for maximum passage of PA and polysaccharides from the culture solution through ME and water.

For example, in methyl extract, the PA reaches about 70%, and in this case the amount of oligosaccharides is 10%. During water extraction, the process is reversed, with PA accounting for 6% and polysaccharides for 80%.

**Table 4.4**

**Biochemical analysis of ether and water extract from G.lucidum mushroom culture solution and biomass ( $V_R= C_1-C_2lgM$, $C_1=24.4$, $C_2=4.0$).**

| Fractions | Title of the faction | Amount of the fraction | | MMP | | Mw/Mn | VR max | MM VR max |
|---|---|---|---|---|---|---|---|---|
| | | ME | SE | Mw | Mn | | | |
| 1 | PA | 74 | 6.0 | 180 | 180 | 1.0 | 15.35 | 180 |
| 2 | Hexosa | 11 | 42.5 | 1085 | 1085 | 1.0 | 13.1 | 1085 |
| 3 | Heptosis | 18 | 31 | 1260 | 1260 | 1.0 | 12.15 | 1260 |
| 4 | Oligosaccharide | 3.0 | 11.5 | 4390 | 3600 | 1.22 | | 2430 |
| 5 | Oligosaccharide | 4.0 | 10 | 5450 | 4500 | 1.21 | 10.86 | 4730 |

One point was clarified regarding the quantitative and qualitative aspects of the total yield of polysaccharides. This was due to the carbon sources used in the optimization. For this purpose, the polysaccharide content of the biomass obtained from the fungus *G.lucidum* S-17 (in the example of insoluble fraction) was studied comparatively using glucose (monosaccharide-hexose), rhamnose (monosaccharide - pentose), maltose and Na-KMS. It became clear from the results that the carbon sources used in the optimization cause both quantitative and qualitative changes in both the biomass and the polysaccharides it contains (table 4.5). As can be seen, in all cases, polysaccharides are accompanied by gluco-mannan content, but there are some changes in the quantitative indicators of individual components. This fact may be of interest in determining the qualitative composition of the target product obtained in the future.

**Table 4.5**

**Chemical composition of insoluble polysaccharide fraction in the biomass of *G.lucidum* S-17 using different carbon sources**

| C source | Soluble sugars, % | Protein, % | Ash, % | Monosaccharide composition (%) | | | |
|---|---|---|---|---|---|---|---|
| | | | | Glucose | Mannoza | Galactose | Others |
| Glucose | 80,4 | 7,1 | 0,76 | 60,1 | 17,8 | 15,2 | 6,9 |
| Ramnose | 78,2 | 6,9 | 0,82 | 57,7 | 17,1 | 14,3 | 10,9 |
| Maltose | 77,1 | 6,6 | 0,77 | 56,3 | 20,1 | 17,2 | 6,4 |
| Na-KMS | 75,2 | 6,3 | 0,88 | 62,4 | 15,9 | 15,6 | 6,1 |

### 4.3. Bactericidal and fungicidal properties of polysaccharide fractions of mushroom of the genus Ganoderma

It should be noted that one of the requirements for the practical use of this or that substance is to clarify their effect on microorganisms, especially those that pose a threat to humans, animals and plants. Two approaches are currently used for this purpose, as some researchers have clarified this issue using classical test cultures, while others consider it acceptable to use microorganisms that have recently attracted attention with their opportunism and toxigenicity. In view of the above, we also considered the use of test cultures appropriate to the second approach in the study. For this purpose, bacteria such as Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escerichia coli, Candida albicans, Aspergillus flavus, Mucor hiemalis and fungi such as Penicillium cyclopium were used as test cultures. It became clear from the results that the bactericidal and fungicidal properties of the two fractions were clarified to some extent, and there were some differences in the quantitative indicators of this activity. Both the solubility of the fractions and the sources of the polysaccharide fractions, as well as the biological activity of the test cultures themselves, play a role in this difference (table 4.6).

As can be seen, both bactericidal and fungicidal activity of the soluble fraction is characterized by a higher value than the insoluble fraction.

On the other hand, a comparison of bactericidal and fungicidal activity shows that bactericidal activity is generally relatively high. However, it should be noted that in general, both bactericidal and fungicidal properties of polysaccharides are characterized by weak and moderate levels in the studied method.

**Table 4.6**

**Bactericidal and fungicidal properties of polysaccharide fractions from active producers (according to the diameter of the lysis zone, mm)**

| Test cultures | G.lipsiense S-17 | | G.lucidum S-42 | |
|---|---|---|---|---|
| | H+ | H- | H+ | H- |
| *St.aureus* | 17 | 14 | 21 | 19 |
| *Bac.subtilis* | 19 | 16 | 24 | 22 |
| *Ps.aeruginosa* | 21 | 17 | 22 | 19 |
| *Esc.coli* | 23 | 19 | 27 | 22 |
| *C.albicans* | 16 | 12 | 19 | 17 |
| *A.flavus* | 16 | 13 | 18 | 16 |
| *M.hiemalis* | 17 | 14 | 20 | 18 |
| *P.cyclopium* | 19 | 17 | 21 | 20 |

This weakens the fact that they are important from a medical point of view, and more precisely, their application as an antimicrobial agent. It is true that high antimicrobial activity is an indication of the unequivocal pharmacological activity of the substance, but nevertheless, the characterization of glucoside bonds in the polysaccharide fractions of both strains of Ganoderma fungi selected as active producers has been carried out.

Clarification of this issue is due to the fact that the nature and ratio of glucoside bonds in the materials used in the production of functional products for various purposes (food, feed and medical), in particular polysaccharides, are important. Thus, it is known that in the formation of polysaccharides are mainly involved α - (1; 4 and 6) - D-glucan and β- (1 → 3) - D-glucan, and their ratio in terms of biological, including pharmacological activity of polysaccharides determines its value. Studies to date have shown that 1,3-β-glucoside bonds are more valuable in this regard, as polysaccharides such as lentinan, schizophils, grifolans and griffins, which are currently used in the world for practical purposes and are derived from xylotrophic macromycetes. these communications are predominant. For the reasons mentioned above, it was considered expedient to evaluate the selected strains from this aspect as well. In this regard, it became clear from the research that these bonds have a relative advantage in the insoluble polysaccharide fraction (table 4.7). As can be seen, the relative

**Table 4.7**

**Ratio of glucoside bonds in the polysaccharide fractions**

| | Relative amount of glucoside bonds, % | | |
|---|---|---|---|
| | 1,3-β-glucoside | 1,4- β-glucoside | 1,6- β-glucoside |
| G.lipsiense S-17 (H-) | 38 | 33 | 29 |
| G.lipsiense S-17 (H+) | 29 | 53 | 18 |
| G.lucidum S-42(H-) | 41 | 32 | 27 |
| G.lucidum S-42(H+) | 31 | 50 | 19 |

amount of 1,3-β-glucoside bonds in the soluble fractions is less than in the H-fraction. For example, the relative amount of 1,3-β-glucoside bond in the soluble fraction of *G. lipsiense* S-17 strain is 1.31 times less than in the insoluble fraction of the fungus. A similar indicator is approximately the same in *G.lucidum* S-42, ie

1.32 times less. On the other hand, the relative amount of these bonds is greater in the *G.lucidum* fungus, and this is manifested in both fractions. Thus, it is 1.06 times less for the soluble fraction and 1.07 times less for the insoluble fraction.

It is known that products intended for one purpose or another must meet certain requirements in this area. The fact that the biomass produced by fungi used to obtain polysaccharides is not toxic, at first glance, suggests that they can be used as feed and food additives. One of the requirements for food is that the product is highly digestible in the body. With this in mind, the digestibility of polysaccharide fractions obtained at the end of the study was also studied and its determination was carried out in vitro according to the known method. It became clear from the results that the digestibility of the H-polysaccharide fraction is low compared to the biomass, and that of the H + is high (table 4.8). From comparison of

**Table 4.8**

**Digestibility of polysaccharide fractions and total biomass from the active producers**

| | G.lipsiense S-17 | G.lucidum S-42 |
|---|---|---|
| Biomass | 47,5 | 50,2 |
| H+ | 73,2 | 75,6 |
| H- | 40,8 | 43,3 |

strains selected as active producer it is clear, that the indicators characterizing the digestibility for both fungi do not differ significantly from each other and are relatively close. However, in *G.lucidum* S-42 fungus, both fractions are characterized by more favorable indicators, which allows to note that its polysaccharides, as well as biomass are more valuable.

## FINAL ANALYSIS OF THE RESEARCHES

Fungi are grouped according to their various characteristics, and at the same time they are studied from different angles, one of which is xylotrophs. These include fungi that taxonomically belong to the *Ascomycota* and *Bazidiomycota* divisions as an ecological group and are mainly adapted to live in woody trees.

There is enough information about the spread of xylotrophic macromycetes in Azerbaijan, and now *"their number is 212"*[15]. Although these fungi have been studied as producers of BAS, the study of xylotrophic macromycetes, which are widespread in Azerbaijan as producers of functionally active polysaccharides, is less comprehensive. Therefore, the study of xylotrophic macromycetes from this aspect can be considered as a justified approach.

In this research, mushroom of the genus Ganoderma Karst were selected as the object of study. The basis for this choice is the extensive study of fungal species belonging to this genus as a producer of BAS, their biosynthetic ability differs at the level of the strain, and so on. gave the basis of the features.

Research was first carried out to determine the species composition of Ganoderma mushroom in the territories of Azerbaijan, which differ in biotic and abiotic components, and to create a collection of their strains and distribution of 4 species belonging to the genus Ganoderma (G.adspersum, G.lipsiense,

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[15] Akhundova, N.A. et al. Evaluation by the Oxidase Activity of Xylotropic Macromycetes Causing White Decay.//Advances in Bioscience and Biotechnology, 2019, 10, 179-187.

G.lucidum and G.rezinaseum) were determined in Azerbaijan, 59 strains of them were released into pure culture and their collection was created. One of the main indicators for the use of fungal strains included in the collection as a producer of BAS is that they quickly form more biomass.

For this reason, the initial selection of mushroom belonging to the genus Ganoderma was carried out in this aspect. It was found that the strains tested also differed in this respect, including those with high growth rates, as well as those that are not suitable for use today due to their biomass yield. It is true that fast-growing strains make up 27.1% of those tested, but almost all of them belong to two species (*G.lipsiense* and *G.lucidum*). Selection of *G.lipsiense* S-17 and *G.lucidum* S-42 as active producers, which differ from all strains in terms of biomass origin, is also a logical result of research conducted at this stage.

Specificity of this or that organism is related to its genome, but the abiotic factors of the environment also play a role in its emergence, and their correct identification allows to obtain maximum results. Therefore, studies on the optimization of these parameters for strains selected as active producers are also included in the study.

The amount of biomass obtained during the cultivation of strains selected under optimized conditions for all parameters under DB conditions for 5 days was 8.0-8.6 g / l. Comparing the obtained results with known strains, it became clear that the selected strains are promising, and their use for this purpose allows to obtain favorable results.

Comparative study of FB and VM belonging to the species selected as the active producer was also carried out, the main criterion of which was the activity of metabolites formed by them with biological activity. It was clear from the results that metabolites with biological activity were found in both water and alcohol extracted parts of both FB and VM, which were naturally formed by strains selected as high-growth producers, but their activity was different. Thus, the biological activity of *G.lucidum* S-42 strain, which is selected as an active producer, is always observed in all variants with an increase. In *G.lipsiense* S-17, the increase and decrease compared to the original is not significant. Thus, in all cases, there is no significant change in the number of infusors compared to the control option. In short, selected strains of fungi of the genus Ganoderma, which are selected as active producers, contain metabolites with similar properties in both FB and VM, and their activity is higher in *G.lucidim*. At the end of research, the specific gravity of polysaccharides in the biomass formed by fungi of the genus Ganoderma and the possibility of its use were clarified. Clarification of this is an important task today, at least because fungi are now one of the main sources for the production of anti-vitreous activity, and on the basis of such drugs are polysaccharides with functional activity synthesized by (mushroom) fungi and abundant 1,3-β-glucoside bonds. stops.

Studies on the evaluation of *G.lucidum* S-42, a fungus of the genus Ganoderma, which is widespread in Azerbaijan and has been selected as an active producer and synthesizes both FB and VM biologically active metabolites, have shown that the biomass it produces 67.4% are polysaccharides. 1/12 of these polysaccharides are soluble fractions and the rest are insoluble fractions. Although the fractions have quantitatively different quantitative indicators, they both have bactericidal and fungicidal activity. It is true that in this case there are some differences in the quantitative indicators of activity, but both fractions are characterized by a large amount of relative 1,3-β-glucoside bonds. In addition, polysaccharide fractions have a higher digestibility than the total biomass. All this suggests that the studied fungus is a promising source as a producer of polysaccharides.

## RESULTS

1. As a result of research carried out in the territories of Azerbaijan differing in natural soil-climatic conditions, the distribution of Ganoderma species such as *G.adserbsium*, *G.lipsiense*, *G.lucidum* and *G.rezinaseum* were determined and the registered species were distributed on separate units and substrates. Although they differ in the lifespan of the fruit body they form, their ecological and trophic relationships, and their synthesis of metabolites with biological activity, they are all characterized by white rot in nature[1, 4-5, 9, 10, 15-16].
2. It has been established, that one of the factors influencing the growth rate of strains of fungi belonging to the genus Ganoderma that form a multi-fruiting body is the lifespan of the fruit body formed by them under natural conditions. Thus, cultures separated from fruit bodies of the same species that are 3 or more years old have a higher growth rate[12, 14].
3. 27,1% are characterized as fast-growing, 35,6% as medium-growing, and 37,3% as late-growing strains of the 59 strains isolated from the genus Ganoderma, among which the highest growth rate is *G. lipsiense* S-17 and *G.lucidum* S-42 strains, and it is relatively convenient to cultivate in a liquid glucose-peptone nutrient medium for this feature to occur, but in this case the amount of glucose added to the medium is 9.5-9.7 g / l. peptone 3.0 g / l, $NH_4NO_3$ - 1.5 (more precisely, 0.037-0.038% for nitrogen), cultivation temperature $28^0$C, initial acidity of the environment 5.5, and cultivation within 5 days[4, 6, 8, 9, 12, 14, 16].

4. It has been established that the parts of strains selected as producers with high growth rates, extracted with water and alcohol from both the naturally formed fruit body and vegetative mycelium, have biological activity. In this case, the biological activity of *G.lucidum* S-42 strain, selected as the active producer, is always increased, while the increase and decrease observed compared to the initial sample taken in *G.lipsiense* S-17 is not significant and in all cases is less than the control variant. A similar situation is observed in metabolites secreted by the latter strain into the culture solution[1, 11].
5. Polysaccharides make up 67.4% of the biomass formed by the *G.lucidum* S-42 fungus, which has a high activity of both the fruit body and the vegetative mycelium, as well as the culture solution, of which 1/12 is soluble and the rest is insoluble polysaccharide fraction. In addition to having quantitatively different bactericidal and fungicidal activity, both fractions are characterized by high digestibility relative to biomass and a relatively large number of 1,3-β-glucoside bonds, which are considered pharmacologically valuable. also shows, that it is promising as an important source for the purchase of medical products[1-3, 6-7, 13].

## LIST OF WORKS, PUBLISHED IN CONNECTION WITH THE DISSERTATION

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