REPUBLIC OF AZERBAIJAN

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ABSTRACT
of the dissertation submitted for the degree
of Doctor of Philosophy

GROWTH CONDITIONS OF GANODERMA LUCIDUM (CURTİS) P. KARST.FUNGI, ITS INTRASPECIFIC POLYMORPHISM AND ANTIMICROBIAL ACTIVITY

Speciality: 2430.01- Mycology

Field of science: Biology

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BAKU -2022

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INTRODUCTION

The degree of significance and study of the topic.The kingdom of fungi (Mycota) consists of agroup of highly diverse heterotrophic eukaryotes. The presence of a chitin on the cell wall, loss of phagotrophic properties, having a complex multicellular mycelial structure that can form a macroscopic structure starting from simple single-celled organisms are characteristic features of fungi.*"Fungi emerged as a 'Third Kingdom' of eukaryotes that are outside the classical dichotomy of animals and plants."*[1] and this "emergence" was "*formalized*"[2] by Whittaker in 1969. However, the taxonomy of this group of organisms, that is, fungi, is characterized by extreme dynamism and only recently genomics and phylogenomics have come to a head in a senseand according to modern data, fungi can be divided into "*9 groups*"[1] (clade) at the phylum-level, one of which is Basidomycota.

Fungi belonging to this group differ from other groups both in terms of the number of species and their ecological functions.There are both microscopic and macroscopic ones, the number of which is *"more than 31 thousand."*[3] Interest in these fungi, especially their xylotrophic species, has been growing in recent years. This is due to the research which has shown that "*mushrooms are a generous and natural source as active producers of nutritional and medicinal compounds"*[4].

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[1] Naranjo-Ortiz, M.A., Gabaldón, T. Fungal evolution: diversity, taxonomy and phylogeny of the Fungi// Biological Reviews, -2019, v.94, iss.6, -p.2101-2137

[2] Whittaker, R.H. New concepts of kingdoms or organisms. Evolutionary relations are better represented by new classifications than by the traditional two kingdoms.//Science, 1969, v.163, -p.150–160.

[3] Coelho, M.A. et al. Fungal sex: the basidiomycota// Microbiol Spectrum, 2017, 5(3): FUNK0046-2016. doi:10.1128/microbiolspec.

[4] Wang, P.-C. Anti-diabetic polysaccharides from natural sources: A review./ P.-C. Wang, S.Zhao, B.Y.Yang[et al.]// Carbohydr. Polym., 2016, 148, 86–97.

On the other hand, the number of producer-fungi studied in this direction so far, considered promising as bioactive compound producers is small and mainly covers several species of fungi, one of which is the genus *Ganoderma*P.Karst.

Ganoderma P.Karst is one of the most important medicinal species, synthesizing a large number of secondary metabolites with different effects and functions.It should be noted that *"phytochemical studies conducted over the last 40 years have allowed the isolation of 431 secondary metabolites from fungi of this genus, among them are biologically active compounds such as polysaccharides, proteins, enzymes, aldehydes, ketones, esters, phenols, steroids, alkaloids, etc."*[5] . The use of these compounds in both nutritional and medicinal purposes is a promising and significant source in today's world in terms of eliminating food shortages.

G.lucidum (Curtis) P.Karst fungus of this genus is the most common. Other fungi of this genus (*G.lipsiense* (= *G.applanatum*), *G.resaceum*, *G.sinense*, etc.) are also studied in this aspect. The studies confirmed their synthesis of one or another secondary metabolite, yet relatively weak. The synthesis of practically important metabolites by both *G.lucidum* and other fungi of the genus *Ganoderma* can at the same time allow to continue their research, as well as to obtain new specific metabolites. In a nutshell, the study of this genus as a producer of bioactive compounds (BAC) is a topical issue, both scientifically and practically.Despite geographyof research centres, nor according to the nature of the research conducted it is not correct to consider the level of research of *G.lucidum*, which is characterized by wide diversity, to be unequivocally high. Thus, in the research conducted on the type of fungus mentioned today, there are many points that are important to

[5] Baby, S., Johnson, A.J., Govindan, B. Secondary metabolites from Ganoderma //Phytochemistry, 2015, v.114, p.66-101

clarify both from a scientific and practical point of view, and the following can be noted.

First, one of the characteristics of *G.lucidum* is the widespread observation of intraspecific polymorphisms, but to date it has not been determined whether this is in any way related to their biosynthetic capability;

Second, the fact that the bioactive compounds synthesized by different biotopes of the fungal strains belonging to the genus *G.lucidum* differ both quantitatively and qualitatively, this issue also varies depending on the conditions of their cultivation, which allows us to note that the probability of finding high-yielding strains from known ones can be realized;

Third and lastly, fungi are one of the groups of living organisms that are relatively little studied and are increasingly in the focus of research in various aspects (mycological, microbiological, biotechnological, phytopathological, genetic-molecular, etc.). This indicates that there is a wide range of possibilities for the separation of strains with higher biological activity in areas that have not yet been studied.

In the light of this, if we focus our attention on the Republic of Azerbaijan, first of all, it should be noted that nature is characterized by richness and diversity in various aspects in the territory of our country and research on the distribution of fungi has been conducted since the second half of the 19th century, and thousands of species have been identified so far. Since the second half of the 20th century, more precisely since the 70s and 80s, not only the species composition of fungi, but also their physiological and biochemical properties have become the subject of research. Those years, the research focused on determining the ability of fungi to synthesize one or another enzyme, if it caused certain diseases in plants, and its use as a biological agent in the bioconversion process.These studies continue today, and at the same time, since the beginning of the second decade of the present century, the study of fungi as producers of various (polysaccharides, polyacetylenes, and antibiotics) BAC has commenced.G.lucidim is also among the fungi studied in this direction. Although this fungus has been studied as a producer of hydrolytic enzymes and polysaccharides, research in the above aspects is weaker and is open to research for all the three reasons.

Objectives and tasks. The purpose of this work is to study the cultivation conditions, intraspecific polymorphism and antimicrobial activity of *Ganodermalucidum* (Curtis) P.Karst, which grows in Azerbaijan.

In order to achieve this, it was considered expedient to implement the following tasks:

- Distribution, frequency of occurrence of *G.lucidum* fungus in Azerbaijan and creating a collection of its different strains;

- Assessment of polymorphism in the fruiting body and vegetative growth phase mycelium of *G.lucidum* fungus, which is registered in Azerbaijan;

- Considering morphological features of fruiting body and vegetative growth phase mycelium, quantitative assessment of biomass obtained during the cultivation of different fungal strains in a liquid nutrient medium;

- Considering morphological features of fruit body and vegetative growth phase mycelium, evaluation of extracellular and intracellular metabolites obtained during cultivation of different fungal strains in liquid nutrient medium for antimicrobial activity;

The research methods. The methods applied in mycological studies are widely used in the research**.** Thus, the mapping technique is used in the research conducted both in the world and in Azerbaijan, in the study of the species composition of macromycetes and their physiological and biochemical properties, the cultivation of pure cultures of

*G.lucidum*was carried out according to the quantitative indicator of biomass yield in a liquid nutrient medium, the toxicity of the metabolites with the biological activity they synthesized was based on the effect of the ability of plant seeds to germinate and the viability of cilia, and bactericidal and fungicidal activity was carried out according to the disc-diffusion method.The presence of at least 4 repetitions of experiments in research also allows for statistical processing of the results. In all cases, the use of results in the dissertation, mainly with an average statistical deviation of less than 5%, can be considered as confirmation of the accuracy of the provided.

The main provisions for defense are:

- Rich nature of the Republic of Azerbaijan created the basis for the widespread of fungi and their active participation in the process of degradation of forest ecosystems;
- Although fungi belonging to the genus *Ganoderma* P. Karst are represented by 4 species in the xylomycobiota of the Azerbaijani nature, 2 of them are characterized as common;
- Despite beingunevenly distributed in forests in ecologically different areas of Azerbaijan, the *G.lucidum* fungus of the genus *Ganoderma*P.Karst, intra-species polymorphism is observed in all of them;
- Intra-species polymorphism also plays a role in the cultivating conditions and antimicrobial activity of fungi of the genus*G.lucidum.*

Scientific novelty of the research. As a result of the research, in the economic regions(according to the 2003 division) of Azerbaijan, such as Guba-Khachmaz (GKh), Sheki-Zagatala (ShZ), Aran (ArN), Ganja-Gazakh (GG) and Lankaran-Astara (LA), the distribution of 52 species of xylotrophicmacromycetes was determined, which differ in hyphal systems, the colour of the decay, they cause under natural conditions,substrate interaction and the frequency of occurrence, of which 7.7% belong to the genus *Ganoderma*.

Ganoderma is represented by species such as *G.aspersum*, *G.lipsense, G.lucidum and G.resinaseum* in formation of xylomycobita of the studied areas and they all have a trimitic hyphal systems, they naturally cause white rot, do not have substrate specificity, are polytrophic in terms of ecological-trophic relations, but they differ in the shape, colour, etc. of the fruiting body they form in natural environment.

Despite the fact that all species of *Ganoderma,* in Azerbaijan, are of medicinal importance,*G.lucidum*fungus is more observable of the polymorphism of the fruiting body formed in nature, as well as its synthesis of metabolites with one or another biological, including pharmacological activity determined to be different at the strain level.

The distribution of *G.lucidum* in ecologically different areas of Azerbaijan is uneven and the natural climatic conditions and flora of the Greater Caucasus are more favourable for its spread.

The fruiting body formed by the *G.lucidum* fungus under natural conditions has a polymorphism; they can be divided into 3 polymorphic groups, which are characterized by their form as polypore, cap and transitional.The differences between the groups are also preserved in the cultural and morphological features of the strains belonging to these groups.

It was found that a liquid glucose peptone medium containing 9.5 g / l glucose, 3 g / l peptone, 0.036% (for nitrogen) NH_4NO_3 is more suitable for obtaining maximum biomass from strains of *G.lucidum* belonging to different polymorphic groups.To do this, the temperature during cultivation should be 26-28°C, the initial pH of the medium between 5.5-5.7, and it was also determined that it is expedient to use 5-day biomass of mushrooms grown in GPLM as planting material.

It was found that the first group of strains belonging to the polymorphic groups of the genus *G.lucidum*, that is, strains isolated from fruiting bodies that are characteristic of cap fungi differ from other groups due to their growth rates, biomass production, low toxicity, as well as high antimicrobial (antibacterial and antifungal) activities.

Theoretical and practical significance of the research. For the first time, studies have shown a link between intraspecific polymorphism and antimicrobial activity of the fungus *G.lucidum*, which is the basis for more efficient use of these fungi for practical purposes in the future.

In the course of research, the future use of *G.lucidum* mushroom in the production of various food and medical products and supplements is promising for both economic and environmental considerations, and the information obtained about them is useful as a basis for organizing the production of biologically active substances of fungal origin in Azerbaijan.

Publication, approbation and application of the dissertation. Therehave been published 14 scientific works on the topic of the dissertation, 11 of which are scientific articles. Dissertation materials were presented at the international conference "Development of science in the 21st century" (Ukraine R., Kharkov, 2018), at the scientific conference "Actual problems of modern biology" (Baku, 2019) and at the First International Scientific and Practical Conference on the subject of "Fundamental and applied scientific research: current issues, achievements and innovation" (Russia F., Penza, 2021).

The organization where the dissertation work is carried out. The dissertation work was carried out in the Laboratory of Biologically Active Substances and Microbiological Biotechnology of the Institute of Microbiology of ANAS.

The structure and scope of the dissertation. The dissertation consists of an introduction, literature review (1st

Chapter), materials and methods (2nd Chapter), experimental part (3rd and 4th Chapters), final analysis of research results, conclusion, practical recommendations, list of used literature and a list of abbreviations, including a total of 210,400 signs.

CHAPTER 1

XYLOTROPHIC MACROMYSETS: TYPE OF COMPOSITION, DISTRIBUTION, CULTIVATION, POTENTIAL AS A BIOACTIVE COMPOUND PRODUCER AND THE PLACE OF GANODERMA LUCIDUM MUSHROOMS AMONG THEM

Section 1.1 of the dissertation analyses the literature of fungi kingdom, their systematics, species composition and ecological functions in nature.

Section 1.2 of the dissertation summarizes the information on the species composition of fungi belonging to the genus Ganoderma P. Karst, their use in biotechnology and the species distributed in Azerbaijan.

Section 1.3 of the dissertation analyses the information related to the study of G.lucidum as a producer of bioactive compounds and clarifies the shortcomings in the study of the fungus.

CHAPTER 2

MATERIALS AND METHODS OF RESEARCH

2.1. General characteristics of the studied areas

The research was conducted in 5 ERs of the Republic of Azerbaijan, such as ArN, LA, GKh, ShZ and GG, which are ecologically different. The selection of these areas for research is due to the fact that "*the forests of the Republic of Azerbaijan are most widespread in these areas"*[6]and the most common areas of fungi selected for research are forests.

2.2. General characteristics of the methods used for analysis

During the research conducted during 2016-2020, 756 FB belonging to xylotrophicmacromycetes were registered in 5 different ER of Azerbaijan, 14 of which were specific to the *G.lucidum* fungus. The collection of FBs was carried out in accordance with the *"mapping technique",*[7] the collected FBs were certified on site and packed for laboratory research. Using the information obtained in the laboratory, the identification of the fungus was carried out according to *"known identifiers".*[8],[9],[10]
Obtaining pure cultures of *G.lucidum* according to *"known methods"*[11], was carried out using malt-extract agar (MEA-2-4B^0) as a nutrient medium.

Cultivation of mushrooms in the vegetative growth phase was carried out in both solid (SSF) and liquid (LSP) state fermentation. Cultivation in SSF was carried out mainly during the separation of fungal cultures, study of cultural-morphological features and determination of growth

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[6] Məmmədov Q.Ş., Xəlilov M.Y. Azərbaycanmeşələri. Bakı: "Elm" nəşriyyatı, 2002, 472 s

[7] Мухин, В.А. Биотаксилотрофных базидиомицетов Западно-Сибирской равнины. Екатеринбург, 1993, 231с.

[8] Мухин, В. А. Полевой определитель трутовых грибов./Б.А.Мухин. -Екатеринбург, -1997, -104 с.

[9] Bernicchia A. Polyporaceae s.//Fungi Europaei., 2005, v.10, 808p.

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

[11] Методы экспериментальной микологии/Под.ред. Билай В.И. Киев: Наукова думка, 1982, 500с.

coefficient, and in LSF conditions during the study of physiological-biochemical characteristics of cultures.

Environmental optimization for strains selected for biomass yield based on generally accepted principles and the main parameters was carried out using P.ZMuradov's*"methods and approaches used in his work."*[12]

Exogenous and endogenous metabolites of fungal cultures were carried out in accordance with the following. First of all, biomass of mushroom cultures grown in DCunder LP conditions for 5 days is separated from the solution so that the released solution, i.e. culture solution (CS) is a source of exogenous metabolites (ExM), and biomass or vegetative mycelium (VM) of endogenous metabolites EnM) is taken as the source. Directly from CS, VM is washed 3 times with neutral phosphate buffer and a certain amount (50 ml) distilled sterile water for 3 minutes. Then it is passed through a tissue shredder 3 times and the crushed part of the biomass is precipitated by centrifugation. The resulting solution is used in research as a source of endogenous metabolites.

The toxicity of CS and VM was assessed for their effect on both seed germination and cilia viability.The *"methodological approaches"*[13] applied in the work of Bakhshaliyeva and others were used to identify both processes. The determination of the

[12] Muradov P.Z. Changes in the enzymatic activity of xylotrophic fungi in the process of bioconversion of plant wastes. B.e.d. abstract of the dissertation. Baku, 2004, 48p.[1313]Bakhshaliyeva, K. et al. Assessment of the prospects of studying and using mushrooms of Azerbaijan as effective producers of biologically active substances // Periódicotchêquímica (Brazilia), 2020, v.17, № 34, p.403-411

[13] Bakhshaliyeva, K. et al. Assessment of the prospects of studying and using mushrooms of Azerbaijan as effective producers of biologically active substances // Periódicotchêquímica (Brazilia), 2020, v.17, № 34, p.403-411

antimicrobial activity of ExM and EnM as the object of study was determined according to the *"disk-diffusion method."*[14]

Under SSF conditions, the *"growth coefficient (GC)" of the fungus was determined according to the formula GI = DHS / T, where D*[15] is the diameter (mm) of the colony formed by the fungus in MEA, H is the height of the colony (mm), S is the density of the colony (visually assessed on a 5 scoring scale) and T is the cultivation period (days).

Having posed in the course of research and being aimed at achieving quantitative results all studies were placed in at least 4 repetitions. The results are *"statistically processed"*[16] and standard mean square deviation was mainly assessed according to the accuracy of the obtained results.

CHAPTER 3

DISTRIBUTION OF GANODERMA LUCİDUM(CURTİS) P. KARSTMUSHROOMS IN AZERBAIJAN and THE FREQUENCY OF OCCURENCE,CREATION OF A COLLECTION OF THEIR CLEAN CULTURES

3.1. General xylomicobiota of the studied areas and the role of fungal species belonging to the genus *Ganoderma*P.Karstin its formation

The initial phase of the research covered all xylotrophicmacromycetes distributed in the study areas, and as

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

[15] Бухало, А.С. Высшие съедобные базидиомицеты в чистой культуре./ А.С.Бухало.-Киев: Науковадумка, -1988, -176 с.

[16] Кобзарь, А. И. Прикладная математическая статистика / А. И. Кобзарь - Москва: ФИЗМАТЛИТ, - 2006, - 816 с

a result, 52 species of 29 genera were recorded. Some of the registered genera are represented by 1, some by 2, and some by 3 and more species. For instance, studies have identified 7 species of the genus *Phellinus*, 6 species of the genus *Trametes*, 5 species of the genus *Fomitopsis*, and 4 species of each genus *Ganoderma* and *Inonotus*. The recorded species are characterized by a wide variety of substrate interaction, ecological-trophic relationships, and the colour of the decay they cause, and the hyphal systems, which makes it possible to note the differences in the ecological functions they perform in nature.

Some of the fungal species recorded in the course of the study are represented in the xylomycobiota with more genera, one of which is the genus Ganoderma with 4 species. These fungi are identical in a number of respects, i.e.their hyphal (trimitic) systems, the natural decay colour(white), substrate interactions (eutrophic), and ecological-trophic relationships (polytrophic).However, they differ in the frequency of occurrence and the visual appearance of the fruiting body (FB). (shape, colour, length, width and thickness, location on the substrate, etc.)

3.2. Research areas of *Ganodermalucidum* and general characteristics of the distribution in substrates

The study of *GanodermaP.Karst* as one of the BAC producers has scientific and practical importance. In accordance with the purpose of the study, the possibilities of using *G.lucidum* mushroom belonging to the genus *Ganoderma* as a producer of BAC have been clarified. It was found that this type of fungus is not widespread in Azerbaijan and its distribution in the studied areas is different (Table 3.1). As can be seen, it is prevalent in the forests of GKhER versusArN, GG and LA forests.

Table 3.1

General characteristics of the prevalence of *G.lucidum* fungi recorded in studies

Studied territories	Number of registered FB	Number of strains released into pure culture	Record of propagated substrates
Gkh	8	7	6
ShZ	3	2	2
ArN	1	1	1
GG	1	1	1
LA	1	1	1
Total	14	12	6

Basidiomycota has the ability to bear FB under natural conditions, but its reserves are limited. To solve this problem, their vegetative mycelium is used. Therefore,12 pure cultures of the *G.lucidum* were obtained during the study (Table 3.1).The recorded pure cultures also differ according to the substrates, and they are distributed in 6 tree species (common hornbeam, common linden, chestnut oak, beech, common poplar and common ash), but common hornbeam has been found to be more suitable substrate for fungi. Thus, 42.9% of the FBs belonging to the fungi registered in the hornbeam.The same can be said about Gkh ER, as 57.1% of the registered FBs were taken from the forests there.

CHAPTER 4

EVALUATION OF MORPHOLOGICALLY DIFFERENT STRAINS SEPARATED FROM FRUITING BODY OF GANODERMA LUCIDUM MUSHROOM ON BIOMASS PRODUCTION AND ANTIMICROB ACTIVITY

4.1. Characteristics of Ganodermalucidum, morphological appearance of its fruiting body formed in nature

Although the lifespan of FBs of *G. lucidum* is annual, they differ in their visual appearance. It means, fruiting body collected in the study can be divided into 3 groups.As can be seen, group 1 (A) includes those that resemble true cap mushrooms, group 2 (B) that adheres to the substrate in the form of a crescent like aphyllophoroid fungi, and group 3 (C) is between these two forms has short legs and large caps (Fig. 4.1). Total 12 released pure cultures, 6 belonged to group A, 3 to group B and 3 to group C.

A B C

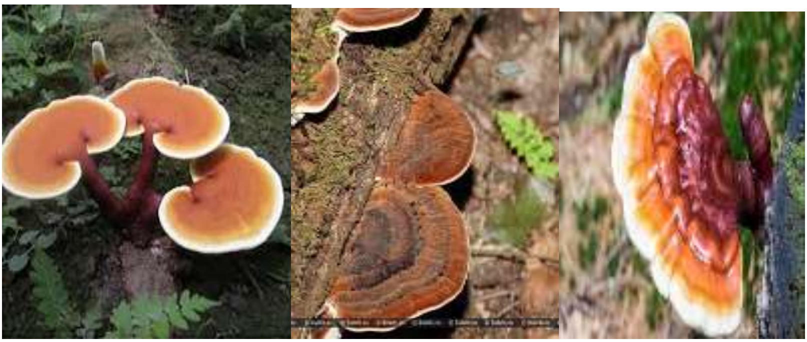

Figure 4.1. General appearance of the FB belonging to the polymorphic groups characteristic of G.lucidum

It became clear from the research conducted on the study of cultures of different polymorphic groups in physiological and biochemical aspects that strains belonging to polymorphic groups are characterized by different indicators both in terms of the amount of biomass they form in a liquid nutrient medium and the rate of growth in standard nutrient media (tabl. 4.1).

Table 4.1

Evaluation of strains of G. lucidum belonging to FB polymorphic groups on biomass yield and growth rate

FB polymorphic groups	biomass yield (5 days, DC, q/l)	Growth rate (MEA, 5 days, mm/day)	Numbers of selected strains	Selected strain indicators	
				biomass release	Growth speed
A	5,7-7,7	7,5-10,1	AG–1	7,7	10,1
			AG-2	7,0	9,5
B	5,4-6,7	6,5-8,9	BG-3	6,7	8,7
			BG-4	6,6	8,5
C	5,3-6,5	5,4-7,9	CG-5	6,5	7,7
			CG-6	6,0	7,4

This difference is observed both within the group and at the intergroup level, and although the clearly expressed dependence is not fully observed, the general trend is that both biomass output, as well as strains belonging to polymorphic group A have a relatively high rate of growth; group C strains are characterized by the lowest rate; group B strains are located between the two of them, i.e. between the polymorphism of the FB and the growth of fungal strains are observed. Therefore, 2 strains with the highest performance were selected from each group and *G.lucidum* is named as AG - 1; AG-2; BG-3; BG-4; CG-5 and CG-6.

Characterizing the colonies formed during the cultivation of the selected strains in the MEA according to the cultural-morphological features, it became clear that despite having many commonfeatures in all 3 groups,there are some differences in morphological features bothin the GI and in the structural elements that ensure the formation of the colony. For example, the growth factor in polymorphic group A is 45,6-50,4, and in groups B and C it is 43,6-47,3 and 40,5-44,5, respectively. In other words, the polymorphism observed in the FB manifests itself, albeit weakly, in the phase of vegetative growth to one degree or another.

4.2. Finding the optimal environment for biomass output for strains of *Ganodermalucidum* belonging to polymorphic groups

Although this or that feature is an indicator associated with the genome of biological objects, including fungi, environmental factors also play a role in its development.Therefore, in the course of research, studies have been conducted on optimization based on factors such as sources C and N, pH, temperature, method and duration of inoculation preparation, cultivation period and the use of glucose-peptone liquid nutrient medium (GPLM) was considered expedient.

4.2.1. Influence of carbon sources on biomass yield of strains belonging to polymorphic groups of *G. lucidum* fungus

Research in this area has shown that mono- (fructose, galactose, xylose, rhamnose), di- (maltose, sucrose and lactose), oligosaccharides and poly-saccharides (xylan) are used as sources of carbon. Biomass yield is generally reduced in the mono-poly direction in all used strains. Thus, when adding oligosaccharides to the environment, the amount of biomass formed during the use of glucose used as a control is almost 2 times less (1.97-2.03), and this is confirmed in all strains. There is a decrease in biomass yield due to the increase in the degree of polymerization of carbohydrates used as a source of carbon. For example, in the sample of *G.lucidum* AG-1 strain, the biomass yield of fructose is 1.09 times, 1.49 times and 1.84 times less, respectively, compared with maltose, oligosaccharides and polysaccharides.

It should be noted that the carbohydrates used in research, i.e. carbon sources, both heptoses and pentoses consist of compounds belonging to different degrees of polymerization. Although heptoses are better absorbed by fungal strains, but in all cases no carbon source can produce a growth effect compared to glucose used as a control,that is, in all cases, glucose is recorded as a more suitable source of carbon.For this reason, it was considered appropriate to use glucose as the only source of carbon in the next phase of the research.Its amount added to the environment was 9.5 g / l, which was determined experimentally in the course of research.

4.2.2. Influence of organic and inorganic nitrogen sources on biomass yield of strains belonging to polymorphic groups of *G. lucidum* fungus.

The results obtained during the clarification of this issue shows that changes in the amount of both mineral and organic nitrogen sources affects the biomass yield in all studied strains and a quantitative indicator of the characterization of this effect increase or decrease depending on both the form of nitrogen (nitrate or ammonium) in the mineral nitrogen source and the biological characteristics of the strains. However, the changes observed in both cases are mainly quantitative. This indicates that the polymorphic grouping is stable at the species level in nitrogen metabolism.

The results obtained on the effect of mineral and organic nitrogen sources on biomass production show that neither NH_4NO_3, nor the effects of peptone yield high results and their concentrations obtained in the initial studies are considered more optimal. Therefore, research has been conducted to clarify the impact of mineral and organic nitrogen sources on the process. It was found that a 1.1-fold reduction in the initial quantity ratio leads to a 1.05-fold increase in biomass output. There is not observed to have a distinctly different effect in the polymorphism of the fungus.

4.2.3. The effect of cultivation temperature and the initial pH of the environment on the biomass yield of strains belonging to the polymorphic groups of the fungus G. lucidum.

The elucidative experiment of these factors was carried out under the condition that the initial pH was in the range of 3-7, and the cultivation temperature ranging between 20-36°C. It was found that for strains belonging to all polymorphic groups, the maximum result in biomass yield is obtained when the cultivation temperature is 27 ± 10°C and the initial pH is 5.7 ± 0.1.

Strains belonging to polymorphic groups do not have a clear dependence on each other both on the cultivation temperature and the initial pH of the environment and almost the same indicator is obtained in the strains of all 3 groups.

4.2.4. Influence of method and duration of preparation of planting material on biomass yield of strains belonging to polymorphic groups of *G.lucidum*fungus.

5 following options were used to prepare the planting material: MEA (2^0B, DC and 5 days), MEA (2^0B, SC, 5 days), LGPM (DC, 5 days), LGPM (SC, 5 days) and MEA (5 days). Planting materials prepared according to options 1-4 are used after crushing in a magnetic mixer and 2 ml of each is added to LGPM with a sterile pipette and cultured at the same period. As a planting material prepared according to option 5, 4 small

square-shaped pieces (0.4x0.4 cm) are added to each flask from a mushroom colony formed in a Petri dish. It is clear from the results that the lowest biomass yield for all polymorphic groups is when using planting material prepared under option 5 (MEA) and the highest with option 3 (LGPM, DC, 5 days). The difference between the lowest and highest is 1.5 fold.

As a result of the research conducted at this stage, it was considered expedient to use LGPM as a nutrient medium for the preparation of planting material, and in this case, the cultivation was carried out under DC conditions, and a more precise optimal variant was selected. In this regard, not only the inoculation of *G.lucidum*, but also the cultivation of biomass under DC conditions was considered optimal.

The physiological condition of the fungal cells that make up the planting material was determined by clarifying the growth phases of the polymorphic groups cultivated in option 3, which was selected as optimal.It was found that strains belonging to all 3 polymorphic groups with small quantitative differences are in the intensive growth phase on days 3-6 of cultivation, and the use of fungal cells in this phase as planting material allows for maximum biomass yield, i.e. it is more convenient to use 4-5 days of biomass of the fungus grown in LGPM under DC conditions as planting material.

4.2.5 Influence of cultivation conditions and duration on biomass yield of strains belonging to polymorphic groups of *G.lucidum* fungus.

According to the above parameters during the cultivation of strains belonging to different polymorphic groups in an optimized nutrient medium, shorter time was required for strains belonging to group A, i.e. 120 hours. This period is 128 hours in group B and 134 hours in group C.

Although some quantitative differences were observed in the studies on finding optimal conditions for biomass production in fungal strains of different polymorphic groups, in general, the indicators required for optimization were similar.

4.3. Toxic activity of strains of polymorphic groups of Ganoderma lucidum

As a result of cultivation of fungal strains in the selected environment for 5 days, VM and CL were considered as target products and evaluated for antimicrobial activity, for which both classical culturetest and toxigenic fungi provided by the laboratory of microbiological biotechnology of the Institute of Microbiology of ANAS were used. Before determining their antimicrobial activity, it was clarified whether the exogenous and endogenous metabolites synthesized by fungal strains were toxic or not. Studies on the germination of seeds show that in some cases, the phenomenon of stimulation is observed, and the final case is only clear form in group A, which is more noticeable in exogenous metabolites (Table 4.4). The results of both endogenous and exogenous metabolites of other groups can be taken as a basis for the absence of toxic effects.

To sum up, regardless of the polymorphic grouping, endogenous and exogenous metabolites specific to *G.lucidum* do not have phytotoxic effects.

A similar opinion can be made on the basis of data obtained during the determination of toxicity of endogenous and exogenous metabolites derived from fungal strains against cilia (Table 4.5). As can be seen, the growth effect is observed in all cases, and this manifests itself in the extraction of dry biomass of fungi with both water and alcohol, and in this case, the activities of polymorphic groups specific to the fungus *G.lucidum* generally repeat the situation observed in phytotoxic activity.

Table 4.4

Determination of toxicity of exogenous and endogenous metabolites synthesized by FB polymorph groups of *G.lucidum* in the vegetative growth phase according to the germination capacity of cucumber plant

	CL(Exogenous metabolites)			VM(Endogenous metabolites)		
	Number of seeds	Number of germinating seeds	Toxic activity	Number of seeds	Number of germinating seeds	Activity
G.lucidum AG-1		328	6,3		320	8,6
G.lucidum AG-2	350	326	6,9	350	318	9,1
G.lucidum BG-3		320	8,6		317	9,4
G.lucidum BG-4		321	8,3		315	10,0
G.lucidum CG-5		316	9,7		312	10,9
G.lucidum CG-6		314	10,3		313	10,6
Control (water)		310	11,4		310	11,4

Table 4.5

The effects of exogenous metabolites synthesized in the vegetative growth phase by polymorphic groups belonging to the fungus G.lucidum on *Tetrahymena pyriformis* viability

	Initial number of ciliate cells	Number of ciliate cells after 24 hours	Growth effect
G.lucidum AG-1	150	295	1,97
G.lucidum AG-2	154	300	1,95
G.lucidum BG-3	160	299	1,87
G.lucidum BG-4	155	294	1,90
G.lucidum CG-5	152	287	1,89
G.lucidum CG-6	158	290	1,84
Control	157	220	1,40

However, when discussing the effect of water and alcohol used for extraction, it is clear that endogenous metabolites in the vegetative mycelium of fungal strains are better extracted with alcohol, and therefore their effect is slightly higher.

4.4. Antimicrobial activity of strains belonging to polymorphic groups of *Ganoderma lucidum*

Researching into antimicrobial activity of strains belonging to polymorphic groups belonging to the fungus *G.lucidum*, high activity of group A strains is evident.Thus, the CS obtained from the strains isolated from the FB, which differ in polymorphism, has antibacterial and antifungal activity, and the main difference is in quantitative indicators (Table 4.6).As can be seen, the highest activity is observed in the strains of the fruit belonging to the polymorphic group A.Group C and Group B have the lowest and moderate quantitative indicators respectively. Interestingly, the case is observed in relation to both antibacterial and antifungal activity.

Study of antimicrobial activity of endogenous metabolites of polymorphic groups of *G.lucidum* fungi selected as the object of study although the general situation is maintained at the time, in this case, the quantitative indicator of antimicrobial activity is relatively low compared to exogenous metabolites

Table 4.6

Antimicrobial activity of exogenous metabolites synthesized in the vegetative growth phase by strains of *G.lucidum*

	Exogenous metabolites		
	AG-1/2	BG-3/4	CG-5/6
Ech.coli	25	23	22
Ps.aeroginaza	23	21	20
St.aureus	24	23	21
A.alternata	22	20	18
C.alpicans	24	22	21
A.fumigatus	21	19	18
F.oxysporium	25	23	21
P.cyclopium	23	20	19
C.herbarium	26	23	21

during extraction with water, during alcohol extraction, it is characterized by almost the same quantity (Table 4.7).For example, the activity of alcohol-extracted metabolites against intestinal spores (Ech.coli) is 22-24,water-extracted 20-22,in exogenous metabolites it is 22-25 mm, these are also characteristic of moderate activity.

Cədvəl 4.7

Antimicrobial activity of exogenous and endogenous metabolites synthesized by G. lucidum strains in the vegetative growth phase (according to the diameter of the lysis zone, mm)

	Endogenous metabolites (1% alcohol)			Endogenous metabolites (water)		
	AG-1/2	BG-3/4	CG-5/6	AG-1/2	BG-3/4	CG-5/6
Ech.coli	24	23	22	22	20	20
Ps.aeroginaza	22	21	21	21	19	17
St.aureus	25	22	21	20	19	18
A.alternata	21	20	19	19	16	15
C.alpicans	23	22	21	21	19	18
A.fumigatus	22	20	19	19	16	15
F.oxysporium	24	22	20	21	20	17
P.cyclopium	22	21	20	20	18	17
C.herbarium	27	25	23	22	20	18

However, both antibacterial and antifungal activity of exogenous and endogenous (alcohol-extracted) metabolites of group A strains of polymorphic groups of the fungus *G.lucidum* are characterized by moderate levels of activity in relation to all culture tests, and this is also the case with endogenous metabolites with a slightly lower quantitative value. All this suggests that the strains belonging to group A have characteristics that can be characterized positively in this respect and shows that their future use for food and medical purposes is more promising.

FINAL ANALYSIS OF THE STUDY

The topic is chosen due to the fact that the selection of this or that substance, primarily the producer of metabolites with biological, including pharmacological activity, is a complex and multi-stage process.This, in turn, is time -and energy -consuming, and demands a lot of materials.

Sometimes fungi differ significantly from each other and according to their morphological features they are characterized as separate species.Elucidation of this issue will allow to obtain information to facilitate the selection process during the initial assessment of fungal strains of the same species in the future.

The solution of this problem began with the determination of the general species composition of xylotrophicmycobiota of forests located in different economic regions of Azerbaijan and the specific place of fungi of the genus Ganoderma in its formation. As a result, 52 species of xylotrophicmacromycetes were found to differ in their hyphal systems, the decay color under natural conditions, their relationship to substrates, and the frequency of occurrence, moreover, Ganoderma P. Karst was found to have 4 species (*G.aspersum, G.lipsense, G.lucidum* and *G.resinaseum*).

Many species of fungi belonging to the genus Ganoderma P. Karst are of medicinal importance, and all 4 species registered in Azerbaijan are considered to be of this type. As a characteristic representative of this genus, G.lucidum fungus contributes to the diversity of the world's research area, it should be considered necessary to study this species more widely in Azerbaijan because of its diversity in the world's research area, wide range of metabolites with synthesized biological, including pharmacological activity, high growth rate of strains isolated from pure culture, fruits they form in natural conditions have more visually different shapes, and due to the fact that the synthesis of metabolites with different functional activity is at least quantitatively different at the strain level.

As a result of research in this area, it became clear that G.lucidum is unevenly distributed in ecologically different areas of Azerbaijan.The natural climatic conditions and flora of the Greater Caucasus, especially the Gkh ER, are more favorable for its spread.Thus, 57.1% of the FB belonging to the fungus recorded in the research fall to the share of forests located in the Gkh ER. This figure is 78.6% for the Greater Caucasus.

The appearance of FBs of the G.lucidum fungus in the form of 3 polymorphic groups was characterized as polypore fungi (C), cap fungi (A) and as transitional forms of these two. during the comparative study of strains isolated from pure culture for each group it becme clear that the level of cultural-morphological features of polymorphism, biomass output,

antibiotic activity etc.manifested itself in one way or another.

These facts will be useful in the selection of active cultures of the fungus (due to characteristics such as biomass yield, antibiotic activity) as an indicator mentioned for the first time, and will greatly facilitate the screening process.

RESULTS

1. The research revealed that in the territory of economic regions of Azerbaijan such as Guba-Khachmaz, Sheki-Zagatala, Aran, Ganja-Gazakh and Lankaran-Astara, there were 52 species of xylotrophic macromycetes of which 7.7% belonged to fungi belonging to the genus Ganoderma P. Karst and which differ in hyphal systems, in the colour of the decay they cause under natural conditions, their interactions with substrates and the frequency of occurrence[5-6, 11, 13].

2. *Ganoderma* P. Karst genus is represented by 4 species (*G.aspersum*, *G.lipsense*, *G.lucidum* and *G.resinaseum*) in the formation of xylomycobita of the studied areas, and they are identical in their hyphal systems (trimitic), the color of their natural decay (white), their relationship to substrates (eutrophic) and their ecological-trophic relations (polytrophic), and differ mainly in the shape and color of the fruit they form naturally[1-2, 6-7, 11, 13].

3. Despite the fact that all species of *Ganoderma* P. Karst in Azerbaijan are of medicinal importance, G. lucidum fungus is responsible for both the diversity of the research area and the wide range of bioactive compounds it synthesizes,the high growth rates,more polymorphism of the fruiting body

formed under natural conditions, its ability to synthesize metabolites with one or another biological, including pharmacological activity with the least quantitatively different strain level make this species to be studied more widely in Azerbaijan[2-3, 7-9, 13].

4. It is evident that the distribution of *G.lucidum* is uneven in ecologically different areas of Azerbaijan, the natural climatic conditions and flora of the Greater Caucasus are more favorable for its spread and the fungi recorded in the studies have a polymorphism of the fruit body formed under natural conditions which can also be divided into 3 polymorphic groups, characterized by their shape as polypore (C), cap (A) and transitional (B), and the difference between them is also preserved in the cultural and morphological features of the strains belonging to these groups[6-7, 12].

5. For the cultivation of strains belonging to different polymorphic groups of the fungus *G.lucidum* liquid glucose-peptone medium is more favorable, in order to conduct this, the amount of glucose in the medium should be 9.5 g / l, peptone as an organic nitrogen source 3 g / l, NH_4NO_3 mineral nitrogen as a source- 0.036% (for nitrogen), the initial pH of the medium is between 5.5-5.7 , it is convenient to use 5-day biomass grown in LGPM as planting material, and the maximum biomass yield of the fungus in these conditions is 120-140 hours[7, 10].

6. The first group of strains belonging to the polymorphic groups of the genus *G.lucidum*, i.e. the strains separated from the fruiting bodies formed characteristic of cap fungi, differs from other groups for the growth rate, the amount of biomass it produces, low toxicity, high antimicrobial (antibacterial and antifungal) activity, and therefore, as a producer of bioactive compounds, it is the basis for the selection of fungi from cultures isolated from this type of FBs[4, 7, 12, 14].

PRACTICAL RECOMMENDATIONS

1. In the search for an active producer of Ganoderma lucidum fungi as a bioactive compound producer, the initial selection should be based on the shape of the fruiting body formed under natural conditions, therefore, the usage of fruiting bodies typical of cap fungi allows to obtain favorable results.

2. When using pure cultures isolated from the fruit body formed by G. lucidum fungus typical of cap fungi as a bioactive compound producer, it is more convenient to carry out the preparation of planting material in a nutrient medium to be used for biomass.

LIST OF PUBLISHED SCIENTIFIC WORKS ON THE TOPIC OF THE DISSERTATION

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The defense will be held on 28 june 2022 at 11-00 at the meeting of the Dissertation council FD 1.07 at the Institute of Microbiology of ANAS.

Address: AZ 1004, Baku, M.Mushfig Street 103

Dissertation is accessible at the Library ofInstitute of Microbiology of ANAS.

Electronic versions of dissertation and its abstract are available on the official website (https://www.azmbi.az/index.php/az/) of the Institute of Microbiology of ANAS.

Abstract was sent to the required addresses on 26 may 2022.

Signed for print:23.0.2022

Paper format: 60x84 1/16

Volume: 37802

Number of hard copies: 20