REPUBLIC OF AZERBAIJAN

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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

PHYSIOLOGICAL BIOCHEMICAL BASIS OF USIING XYLOTROPHOUS MACROMYCETES AS PRODUCERS OF PROTEOLYTIC ENZYMES

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INTRODUCTION

Relevance of the topic and degree of development.

İn modern times, whentechnology is developing on a rising line-population growth is observed with a serious lack of environmentally friendly food, important proteins and minerals in food. Solving these problems and purchasing natural products, providing the world's population with genome-free food, food with protein supplements, meeting the populations needs of biologically active substances have become the most priority areas of science (pharmacology, microbiology and biotechnology).

Making useful and efficient use of plant waste to buy biologically valuable foof products, maintaining a normal ecological environment by reducing its harmful effects on the environment are the main goals of scientific researchers. For this purpose, it is considered more promising to process plant-based waste recycling technology on biological basis, of which "*there are two types, enzymological and microbiological*"[1]. Significantly positive results have been obtained in plant waste as a result of microbiological conversion, which consists in the cultivation of microorganisms, including fungi and their transformation into products of various purposes. The selection of active enzyme producers during the utilization of plant waste by enzymological conversion is the most important condition for obtaining good results. Hydraulic enzymes are the main force of enzymological conversion.

Amiliotic, lipolytic, proteolitic etc with the help of a hydraulic impact, the pathogenic fungi-owner substructor is divided into the texture and transcends the texture of the substato, and the antitheli, protein synthesis mechanism completely. "*Protaineic farms are of*

[1] Машанов, А.И., Величко, Н.А., Ташлыкова Е.Е.Биоконверсия растительного сырья: учеб. Пособие. Красноярск: Краснояр. гос. аграр. ун-т., 2014. – 223 с.

special importance in this process, i.e. in the study of the physiological mechanism of the invasy, the physiological mechanism of virulence."[2] *Alkaline protease, cysteine protease, metalloprotease, aspartate protease, etc enzymes synthesized by fungi, including xylotrophic macromycetes"*[3,4] together with others play an important role in the penetration of the pathogenic fungus into the host plant and in the nutrition of the pathogen. Thus, proteases, play an important role in destroying the protein mass of plant tissue during the settlement of plant's enzyme production and immune system-antibodies (take intoaccount that the particles of the immune system are of protein origin).

İt is clear that *" the mineralization of plant residues and the return of these minerals to the food chain in nature is characterized by the decomposing – pathogenic properties of xylotrophs"*[5]. The existence of a close relationship between pathogenicity and proteolytic enzymes has also been determined.

Due to their economic and environmental advantages, the demand for enzymes in industries is increasing rapidly. Enzymes are widely used in the food industry, light industry, medicine and pharmacology. Among the extracellular hydrolytic enzymes secreted by fungi, those with fibrinolytic and anticoagulant effects are used in the treatment of thrombosis. Therefore, the role of enzymes in pharmacology and drug production is indispensable.

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[2] Yang N, Matthew MA, Yao C. Roles of Cysteine Proteases in Biology and Pathogenesis of Parasites. // Microorganisms,- 2023; 11(6):1397. https:// doi. org/ 10.3390/microorganisms11061397.

[3] Song P, Zhang X, Wang S, Xu W, Wang F, Fu R, Wei F. Microbial proteases and their applications. Front Microbiol. 2023 Sep 14;14:1236368. doi: 10.3389/fmicb.2023.1236368.

[4] Kumar Ch.N., Koreti, D., Kosre, A., & Kumar, A. Proteolytic Enzymes Derived from a Macro Fungus and Their Industrial Application// IntechOpen., 2022. doi: 10.5772/intechopen.102385.

[5] Singh, C., & Vyas, D. Biodegradation by Fungi for Humans and Plants Nutrition.// IntechOpen, 2022. doi: 10.5772/intechopen.99002.

Proteases have also received special attention in industrial fields. Although various sources have been cited "*as producers of these enzymes by various researchers, those obtained from bacteria and fungi are more valuable."*[6] Thus, enzymes obtained from microorganisms differ from other producers due to their economic efficiency, that is, their low cost, high quality, large quantity and wide variety of production. Biotechnology is valued as the only opportunity to create low-waste and environmentally friendly technologies, but there are a number of problems that hinder the efficiency of bioconversion of waste, such as the lack of producers with high biological activity, the lack of physio-chemical indicators of the enzymes they synthesize. İn all of this, it is also possible to note the importance of conducting research in this direction.

The purpose and tasks of the research. The purpose of the presented is to evaluate a number of xylotrophic macromycetes isolated from different biotopes of Azerbaijan according to the activity of proteolytic enzymes and to study some physiological - biochemical features of enzyme synthesis in them.

İn order to achieve the set goal, it was considered necessary to solve the following tasks:

1. Seperation of strains belonging to different xylotrophic macromycetes according to their ecotrophic relations from different areas of Azerbaijan and creation of their collection;
2. Evaluation of the strains included in the collection according to the activity of proteolytic enzymes and selection of active producers;
3. Determination of the type of proteases synthesized by strains selected as active producers and the parameters that regulate the intensive occurence of synthesis in them;
4. Selection of conditions for purchase of partially purified enzyme preparations with proteolytic effect from active producers.

[6] Muthulakshmi, Ch., Gomathi, D., Kumar, D.G. et al. Production, Purification and Characterization of Protease by Aspergillus flavus under Solid State Fermentation// Jordan Journal of Biological Sciences, 2011, v.4, №3, p.137-148.

Research methods. Sampling was carried out according to the systematic and unsystematic route method accepted in mycology. Extraction of fungi into pure culture was carried out according to known methods using WMS as a nutrient medium (2-3°B) and the purity of the culture was carried out by means of a microscope with a magnification of up to 2500 times (OMAX 40X – 2500 X LED Digital Lab Trinocular Compound Microscope). Pure cultures were stored in a refrigerator and transffered to a new nutrilend medium every 4-5 months. During the study of the enzymatic activity of fungi, their cultivation was carried out in a solid nutrient medium, more precisely in a glucose - peptone medium (GPM) under deep cultivation conditions. All experiments were performed in at least 4 replicates and the results obtained were statistically processed.

The main provisions of the dissertation presented for defence:

1. The enzyme system of xylotrophic macromycetes distributed in Azerbaijan includes proteases, but they differ from each other in terms of their activity level.

2. The active strains of the studied species participate in the degradation of the protein mass of plant waste,accelerating the mineralization of organic residues and the occurence of the food chain in nature.

3. Synthesis of different types of proteases in different amounts and proportions in fungi species with high proleotic activity also allows to clarify their ecotrophic affiliation.

4. The fact that xylotrophic macromycetes cause white rot under natural conditions means that their protealytic activity is also high.

Scientific novelty of the research. As a result of the conducted research, 107 strains of 25 types of xylotrophic macromycetes, which are characterized by diversity in the color of the decay caused by the decay, hyphal systems, the shape of the fruit body formed under natural conditions, the duration of life and

drawing, were collected from the natural and artificialforests located in the Great Caucasus, Kura-Araz plain and Talish mountains of Azerbaijan, grown in pure culture and characterized for proteolytic activity.

İt became clear that the level of proteolytic activity of different snake fungi in terms of ecotrophic relationships is also different, and the proteolytic activity of fungi belonging to true biotrophs and polytrophs is characterized by a higher index than that of saprotrophs. Proteases synthesized by xylotrophic macromycetes also differ according to the structure of their catalytic centers, and serine, cysteine, and metallo- proteases are found among the proteases they synthesize, but their ratio varies depending on the fungi. Although there is no clear correlation between the ecotrophic distribution of fungi and the type of proteases, the ratio of different types of proteases is more widely followed in polytrophs and true biotrophs. İn xylotrophic macromycetes selected as active producers of proteolytic enzymes, the synthesis of proteolytic enzymes takes place in a consultive way according to the classical theory (Jakob Mono) and inductive way according to the unitary theory, but the complexation of the composition of the carbon source in the direction of mono-. Di- and poly- saccharides is the cell of the synthesized enzyme affects the excretory part.

When the aqueous extract taken from the substrate where it is settled is added to the environment, more biomass accumulation occurs in Inonotus hispidus fungus, which also leades to an increase in the activity of proteolytic enzymes. On the other hand, during the maintenance of the working culture, the addition of the material taken from the host plant and converted into a nutrient medium leads to an increase in the enzymatic activity, which can be evaluated as the fungus gaining a certain adaptation sign resulting from the biotrophic lifestyle.

Theoretical and practical significance of research. The

obtained results regarding the proteolytic activity of different types of fungi enrich the plants aboutthe enzymatic properties of these fungi.

The complexation of carbon sources in the mono- dipoly direction further increases the synthesis of proteolytic enzymes. The results obtained during screening allow the use of fungi such a *Ganoderma applanatum, Inonotus hispidus*, and *Bjerkandera adusta* as producers of acid proteases.

Applying the obtained results can be of great benefit in preventing environmental pollution, improving the ecological situation, and expanding the raw material base of the microbiology industry.

Dissertation approval and application. 14 scientific works related to the dissertation topic have been published, seven of which are articles and seven are theses. The materials of the dissertation were presented at the international scientific conference on Global Science and innovation (Khazakhistan R., Nursultan, 2020),"On the main problems of quality assurance of university industrial relations" (Ganja 2020),"On research problems of young people during the pandemic" (Ganja 2021), at the international Scientific-practical conference, at the "International scientific conference" (Italy, Rome,2023), "Review of modern science" at the international scientific con (Zurich, Switzerland, 2023), at the XXVII international scientific practical conference (Canada, Edmonton,2022) on "Tendencies of young scientists in the development of science" and reported.

The name of institution where the dissertation work was performed. The dissertation was completed in 2016-2022 at the Microbiological Biotechnology and Biologically Active Substances Laboratory of the Institute of Microbiology of ANAS.

The structure of the dissertation and the total volume taken with the mark. The dissertation consists of an introduction and 5 chapters, a final analysis of the results, conclusions and a list

of used literature. The dissertation consists of a total of 240500 marks, including tables, graphs, and a bibliography.

CHAPTER I
PROTEOLYTIC ENZYMES: BIOLOGICAL FUNCTION, CLASSIFICATION, TYPE, SOURCES OF PURCHASE, BASIC PRINCIPLES OF PRODUCER SELECTION, NATURE OF SYNTHESIS AND FIELDS OF APPLICATION

The functions, classification and importance of proteolytic enzymes in various fields in section 1.1 of the dissertation, the sources of enzymes and the biological basis of producer selection in section 1.2, the catalytic activity of proteolytic enzymes of basidiomycets and a number of biochemical properties of proteases in section 1.3, fungi in section 1.4 the role of proteolytic enzymes in causing pathologies and their caharacteristics according to the type of decay, and information about the importance of proteolytic enzymes and the areas where they are used is given in section 1.5.

CHAPTER II
MATERIAL AND METHODS OF WORK

2.1. The object of research and methods of cultivation of producers

The main object of the research was xylotrophic macromycetes and proteolytic enzymes synthesized by them, which spread in natural and artifical forests located in different areas of Azerbaijan.The removal of fruit bodies of fungi from natural substrats, on-site passporting, identification, extraction. Into pure culture was carried out according to *"known methods and*

determinants accepted in mycology"[7,8,9,10]

At this time, systematic or non-systematic route methods were used in agarized saponin juice (ASJ) as a nutrient medium.

The initial evaluation of the fungi cultures was carried out according to their biomass yield, which was based on the biomass yield (g/l) produced by the (DC) fungi under deep cultivation conditions in dry glucose-peptone nutrient medium (SGPNM) for 5 days. During the study of the enzymatic activity, the biomass and culture solition (CS) produces by fungi during cultivation in the specified nutrient medium were used. Biomass formed during the use of biomass as a source of proteolytic enzymes is broken up in a tissue grinder 3 times in a phosphate butter (3 times, 3 minutes), centrifuged, and the obtained supernatant is used as a source of intracellular (endo) enzymes.

The total proteolytic activity of the cultures was determined by both "*spectrophotometric*"[11] and "*viscometric*"[12] methods. Nacaseianate and gelatin were used as substrats, respectively.

The amount of proteins was also determined by "*spectrophotometric*"[11] method.

All experiments were performed at least 4-6 times and the obtained results were processed "*statistically*"[13].

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

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CHAPTER III
SEPERATION AND CHARACTERIZATION OF XYLOTROPHIC FUNGI DISTRIBUTED IN AZERBAIJAN ACCORDING TO THEIR SPECIES COMPOSITION

3.1. General characteristics of xylotrophic macromycetes isolated from the studied forests

Basidoma samples belonging to 25 species were taken from different areas of Azerbaijan, pure cultures of 107 xylotrophic fungal strains were obtained from them (table.3.1) As can be seen, the number of species belonging to invidual genera, as well as strains, is different. For example, there are 4 species belonging to the genus *Trametes* and 1 species belonging to the genus *Pleurotus*. Their strain number is equal to 20 and 5, respectirely.

At the same time, the recorded fungi species are also characterized by diversity due to some of their characteristics, first of all, their ecotrophic relationships, the color of decay they cause under natural conditions, their hyphal systems, the length of time and weight of the fruit body (FB) they form in nature. For example, *Inonotus hisbidus* is a biotroph due to its ecotrophic relationships, it causes brown rot under natural conditions and the hyphal system is monomitic. Analogous indicator in *Trametes versicolor* fungi is saprotrophic, white and trimitic, respectively.

İn addition, they also differ from the mentioned fungus in terms of their ability to grow in standard nutrient media, which reguires that their growth coefficient be characterized by different indicators. Nevertheless, all the registered fungi are capable of rapid growth, and for this reason, it was considered appropriate to test all of them during screening for the activity of proteolytic enzymes. On the other hand, there is no research material confirming the existence of a clearly expressed dependence between the synthesis of the enzyme and the amount of biomass yield.

Table 3.1.

Taxonomic structure of xylotrophic macromycetes isolated from different forests of Azerbaijan

Class	Row	Family	Genus	Strain
Agaricomycetes	Polyporales	Polyporaceae	Cerrena	1(4)
			Fomitopsis	3(12)
			Fomes	1(4)
			Polyporus	2(7)
			Trametes	4(20)
		Ganodermataceae	Ganoderma	2(8)
		Meruliaceae	Bjerkandera	1(4)
		Fomitopsidaceae	Laetiporus	1(4)
	Hymenochaetales	Hymenochaetaceae	Inonotus	3(15)
			Phellinus	4(16)
	Agaricales	Agaricaceae	Pleurotus	1(5)
			Schizophyllum	1(4)
		Physalacriaceae	Armillaria	1(4)
Total	3	7	13	25 (107)

3.2. Annotated list of recorded fungi

Ann annotated list of 25 species of xylotrophic macromycetes (Basidiomycota) recorded during the research was compiled leased on the following information:

1. The current name and systematic status of the fungi on the official website of the International Mycological Association;
2. The substrate where the fungus was first recorded during research;
3. The color and type of ecotrophic relationship of the decay caused by the fungus in natural conditions;
4. The life span of the fruit body (FB) it forms and some signs to pay attention to during identification.

CHAPTER IV
EVALUATION OF XYLOTROPHIC MACROMYCETES ISOLATED FROM AZERBAIJANI FORESTS ACCORDING TO PROTEOLYTIC ACTIVITY

4.1.Screening of xylotrophic macromycetes for protelytic activity

Based on the analysis of literature data, it was determined that xylotrophic macromycetes found "*more confirmation"*[14] in a dry glucose-peptone rich medim (DGPM).

This fact has been *"confirmed in teh research conducted in recent years"*[15]. For this reason, it was considered appropriate to evaluate the fungi separated in the course of research according to the activity of proteolytic enzymes, that is, to use this medium during screening.

At the initial stage of the screening process, the cultivation of fungi was carried out for 5 days, and from the results obtained, it was clear that the fungal strains differed from each other in terms of their activity level, even at the strain level (Table 4.1.). as can be seen, the difference between the maximum and minimum quantitative indicators of proteolytic activity observed in fungi 2,8 times, in this case, the minimum indicator is observed in *Tratemes zonatus,* and the maximum indicator is observed in *Inonotus hisbidus* fungus strains. The proteolytic activity of the strains belonging to *Armillaria mellea, Fomitopsis pinicola, Fomes formentarius, Ganoderma applanatum* and *Laetiporus sulphureus* was higher than others, and it is interesting that all of them don't belong to saprotrophs in terms of ecotrophic

[14] Muradov P.Z. Bitki tullantılarının biokonversiyası prosesində ksilotrof göbələklərin fermentativ aktivliyinin dəyişilməsi./b.e.d. dissertasiyasının avtoreferatı/-Bakı, 2004, -48s.

[15] Musayeva V.H. Yağ istehsalı tullantılarının utilizasiyasının biotexnoloji əsasları:/ab.ü.f.d. dissertasiyasının avtoreferatı/-Bakı, 2021, -32 s.

relationships, that is, they are biotrophs (*Armillaria mellea, Fomes fomentarius* and *Inonotus hisbidus*) or biotrophy or saprotrophy is not true. İn true saproteophus (*Polyporus varius, Trametes hirsuta, T.versicolor, T.zonata* and *Schizophyllum commune*) the quantitative indicator of proteolytic activity is characterized by a relatively low indicator. This fact itself allows us to note that proteolytic enzymes have a certain indicator quality in the ecotrophic affiliation of fungi.

Table 4.1.

Evaluation of fungal strains belonging to xylotrophic macromycetes according to proteolytic activity.

№	Fungal species	Activity (bv/me)
1	*Armillaria mellea*	0,43-0,63
2	*Cerrena unicolor*	0,41-0,54
3	*Fomitopsis cytisina*	0,40-0,52
4	*F.pinicola*	0,52- 0,65
5	*F.rozea*	0,40- 0,48
6	*F. fomentarius*	0,51-0,64
7	*Polyporus squamosus*	0,26-0,36
8	*P.varius*	0,29-0,34
9	*Trametes hirsute*	0,29-0,40
10	*T. pubecsens*	0,42-0,53
11	*T. verzicolor*	0,25-0,36
12	*T.zonatus*	0,24- 0,32
13	*Ganoderma applanatum.*	0,52-0,63
14	*G.lucidum*	0,38-0,50
15	*Bjerkandera adusta*	0,41-0,53
16	*Laetiporus sulphureus*	0,55-0,62
17	*Inonotus dradeus*	0,38-0,57
18	*İ.hispidus*	0,56-0,68
19	*İ.pini*	0,36-0,54
20	*Phellinus iqniarus*	0,41-0,56
21	*Ph.pini*	0,35-0,53
22	*Ph.pomaseus*	0,36-0,52
23	*Ph.robustus*	0,38-0,54
24	*Pleurotus ostreatus*	0,26-0,43
25	*Schizophyllum commune*	0,32-0,37

İn true saprotrophs (*Polyporus varius, Trametes hirsuta, T. Versicolor, T.zonata* and *Schizophyllum commune*) the quantitative indicator of proteolytic activity is characterized by a relativiely low indicator. This fact itself allows us to note that proteolytic enzymes have a certain indicator quality in the ecotrophic affiliation of fungi. More specifically proteolytic enzymes can be characterized as having an active role in the realization of the parasitic lifestyle of fungi. Considering this idea, in the next stage of research , it was considered appropriate to true biotrophs and polytrophs in terms of ecotrophic relationships in order to clarify the issues related to the synthesis of proteolytic enzymes.

Bioconversion (microbiological and enzymological) of fungal lignocellulosic plant substrates such as *B.adusta* P-40, *C.unicolor* M-3, *P.ostreatus* F-118, *T.hirsute* M-5 and *T.versicolor* D-13, which don't have high activity of proteolytic enzymes conversion and the effectiveness of their use for this purpose in the future is justified.

4.2. Effects of carbon and nitrogen sources on the synthesis of proteolytic enzymes and optimization of the environment

İn the next stage of research, it is considered appropriate to use the strains in the collection as active producers of proteases, and to clarify the issues related to enzyme synthesis in them, to use strains belonging to species such as *Ganoderma applanatum* and *Inonotus hispidus*, as well *as Bjerkandera adusta*, which are selected as active producers of protease has been done. İn order to use these fungi for practical purposes, studies were first conducted on the optimization of the environment required for their maximal synthesis of proteases. Thus, in the course of the research, it was considered appropriate to study the classical parameters of the source of carbon and nitrogen (organic and mineral) the method and duration of planting material preparation, the determination of the

duration of cultivation, as well as the storage conditions of the culture used for planting. Other indicators, for example, the initial acidity of the medium, were specified based on literature data. Thus, in many studies conducted, weak acidic medium, more precisely, nutrient media (natural, synthetic, and semi-synthetic) used for fungi cultivation, pH (between 5-6) after preparation and sterilization, mineral nitrogen source NH_4NO_3, and deep cultivation it was considered appropriate to carry out cultivation (DP) conditions. İt was clear from the results obtained from the studies conducted on the determination of other parameters that the parameters of the environment required for their cultivation are both universal and specific in nature (Table 4.2.). As it can be seen, all three fungi are characterized by the same indicator according to the method and duration of the planting material preparation that is, the biomass obtained during the cultivation of fungi in DC conditions in DGPM for 5-6 days is considered more effective planting material for all three cultures and they are used when the enzyme activity is characterized by a maximum indicator. As a source of organic nitrogen, peptone is more favorable for all three fungi, although in different amounts. The remaining indicators can be attributed primarily to the carbon source and the cultivation period. Proteolytic activity in fungi differs according to qualitative and quantitave indicators of parameters. For example, the addition of glucose as a carbon source to the environment causes a more intense synthesis of proteases in the fungus *Ganoderma applanatum*. A similiar situation is observed in the fungus *Bjerkandera adusta* when sucrose is used. Unlike glucose and sucrose, which are considered favorable carbon sources, *I.hispidus* produces more biomass in the extract obtained from the substrate from which it is separated, which also leads to an increase in the activity of proteolytic enzymes.

Table 4.2.

The main parameters of the optimized environment for the selected fungal strains as active producers of enzymes

№	The name of the spieces	Source and quantity (g/l)		Sow material for use maintenance of culture	Method and duration of planting material preparation	Cultiva-tion period (hour)
		C	Organic nitrogen (g/l) Mineral nitrogen (% nitrogen)			
1	*B.adusta*	Sucrose, 11	Peptone(2,8) NH_4NO_3(0,36)	AMJ(2^0B)	DNPM, 5-6 day, DB	148
2	*G.appplanatum*	Glucose, 10	Peptone 3,0 NH_4NO_3(0,35)	AMJ(2^0B),		140
3	*İ. Hispidus*	Extract prepared from the substrate of the fungi, 10 ml/l	Peptone, 3,2 NH_4NO_3(0,34) or $NaNO_3$(0,34)	Obtained from the substrate from which it üas separated to which soild particles in powder form are added, AMJ(2^0B),		160

One of the parameters where the difference is observed is the storage conditions of the working cultures of the strains belonging to B.adusta, G.applanatum and I.hispidus fungi selected for the next stage of research. As a rule, the storage of basidiomycosis strains is carried out in BMS (2-4°B). This was confirmed in our research, as BMS (2°B) is suitable for maintaining working cultures of *B.adusta* and *G.applanatum* fungi. In *I.hispidus*, the addition of powdered particles from the substrate from which the fungus was isolated to that environment increases the efficiency of the process.

İt should be noted that during the optimization of the environment for enzyme synthesis in fungi selected as active producers, a number of issues were clarified, of which the following should be noted:

1. Although the synthesis of proteolytic enzymes, in fungi takes place in a constitutive way according to the Jakob-Mone theory, but according to the unitary theory, it happens in an inductive way, causes an increase.

2. As a carbon source, unlike glucose and sucrose, which are considered favorable carbon sources, *I.hispidus* produces more biomass in the extrat obtained from the substrate from which it is separated, which also leads to an increase in the activity of proteolytic enzymes. On the other hand, during the Maintenance of the working culture, the addition of the material taken from the host plant and powdered to the nutrient medium also leads to positive results. The reason for this is that, although the fungus belongs to true biotrophs, its biotrophy is ecological in nature. However, despite this, it has also acquired a certain adaption characteristic resulting from the biotrophic lifestyle. This manifests itself with letter growth in host-plant materials.

3. Although its absorption in the form of ammonia as a source of inorganic nitrogen has been determined to be more favorable in many basidiomycetes, this situation does not manifest itself in the

fungus *I.hispidus*, as the fungus can absorb both ammonia and nitrate forms of mineral nitrogen equally.

According to all parameters, they also differ according to the observed period of maximum activity of proteolytic enrymes during the cultivation of fungi in the optimised environment (fig. 4.1). Thus, the *G.applanatum* fungi has maximum activity faster than. The other 2 fungi, while the *I.hispidus* fungi reaches it later. Nevertheless in all cases, even when the other two fungi reached their maximum, the activity level of *I.hispidus* was higher. This situation, in turn, allows us to note once again that proteolytic enzymes play an important role in the process of pathogenesis.

4.3.Physico – chemical and biological properties of proteolytic enzymes

It should be noted that "*proteolytic enzymes are classified according to the structure of their catalytic centers"*[16] and according to current views they are divided into four subgroups: serine-, cysteine-, metallo- and thio-proteases. These proteases differ from each other in terms of the location of active centers and the optimum pH for maximum effect.

Synthesis of proteolytic enzymes synthesized by this or that producer from this aspect is one of the tasks performed in similar works. Taking this into account, it was Considered appropriate to carry out this in fungal strains with relatively high proteolytic activity. As a rule, they use various specific inhibitors for these purposes.

[16] Mótyán JA, Tóth F, Tőzsér J. Research applications of proteolytic enzymes in molecular biology//Biomolecules, 2013, v.8, 3(4). -p.923-942

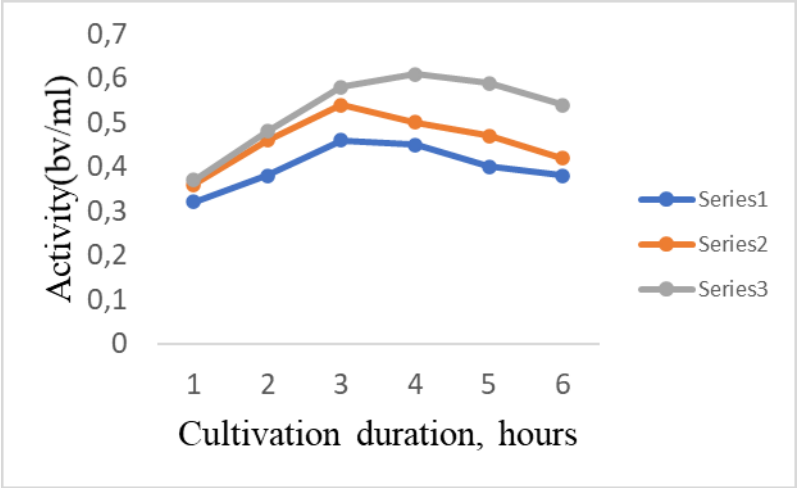

Figure 4.1. Change of proteolytic activity in fungi depending on the duration of cultivation (by 1x48 calculation).

1. *Bjerkandera adusta;*
2. *Ganoderma applanatum;*
3. *Inonotus hispidus*.

For example, for cysteine proteases iodoacetamide (IAA), for metalloproteases Ethylenediamineteraacetic acid (EDTAA), and for serine proteases Phenylmethylsulfonyl – fluoride (FMSF), Chloromethylketone thiosyl – L – phenylalanine (XMKTF) and Chloromethylketone thiosyl – L – lysine (XMKTL) are considered specific inhibitors. Since there is no specific inhibitor for the latter, the simultaneous use of the tree mentioned can be considered sufficient to confirm its presence.

From the obtained results, it became clear that the proteolytic enzymes synthesired by the studied fungi differ from each other from the point of view of the groups, as they show different reactions to the effect of the enzyme inhibitors used (fig.4.3). Apparently, among the proteases synthesized by fungi, all are

found, but their proportion varies depending on the fungus. There is no clearly expressed dependence between the ecortophic division of fungi and the type of proteases, but the Issue is more broadly accompanied by another point, that is, the relative amount of individual types of proteases in the direction of facultative saprotrophy, facultative biotrophy and true biotrophy according to the previous division of fungi. This is a factor that once again strengthens the above-mentioned idea, that is, the connection between proteolytic activity and biotrophy.

It should be noted that the relationship between fungi and bacteria is generally characterized as symbiotic, parasitism and neutralism, but if we approach the issue from a slightly different angle, there is a need for some clarification of this issue. Thus, the characteristic feature of biotrophy is related to meeting the demand for organic matter at the expense of living things. In the case of symbiosis, i.e. cohabitation based on mutual benefit, the demand for organic matter of the fungus is met at the expense of the living organism. On the other hand, due to the effect of a fungus with a biotrophic lifestyle, the life activity of the plant is gradually weakened and destroyed over a long period of time. Neither our studies nor literature data confirm that it doesn’t occur in the sample of hispidus fungus. In

Table 4.3

Effects of inhibitors on the synthesis of exopros theses of xylotrophic fungi.

İnhibi-tors	Solidity, mM	Residual activity %						
		B.adusta	P. ostreatus	G. applanatum	L. sulphureus	İ. hispidus	F. pinicola	T. versicolor
YAA	4	95	92	91	78	65	60	100
EDTST	10	14	80	43	47	51	54	72
FMSF	1	81	60	60	58	53	43	50
XMKTF	3	86	100	98	75	68	60	98
XMKTL	2,5	93	94	96	83	76	72	97
YAA+ EDTST+ FMSF	4:10:1	16	12	19	24	27	25	11

our opinion, this form of relationship can be marked as a transitional form between parasitism and symbiont. More precisely, it would be logical to divide plant-fungus relationships into two places, such as parasitism and neutralism, and characterize them as follows:

- Symbiotrophy that is, a way of living together based on mutual benefit
- Mild parasitism, long term cohabitation with the host plant without serious damage
- Acute parasitism, i.e. cohabitation with severe damage to the host

If we caharacterize the selected fungi as active producers of proteolytic enzymes corresponding to the mentioned division, then I.hispidus and G.applanatum fungi can be characterized as mild parasites.

FINAL ANALYSIS OF THE RESULTS OBTAINED FROM THE RESEARCH

Enzymes, that is, biological catalysts, ensure the realiration of the processes that occur in living things and that this happens at a high speed. For this reason, enzymes more found in species belonging to all taxonomic groups of living things and today the sources of enzymes include plants, animals, and microorganisms (bacteria and fungi). Enzymes that have the same structure and perform the same function, despite being obtained from different sources, are systematized according to the nature of the reactions they catalyze, one of these groups being hydrolases. Proteolytic enzymes are of particular importance among hydrolases characterized by a wide variety length in terms of their number and field of use. Located in various parts of the cell, including the plasma membrane, this enzyme acts as a simple but powerful destructive enzyme necessary for protein catabolism and amino acid synthesis in all living organisms. In addition, proteases play an

important role in a number of biological, physiological and pathophysiological processes in both unicellular and multicellular organisms. This allows us to note that proteases and their producers are a relevant object for research.

Taking this into account, in the presented study, the assessment of macromycetes distributed in the conditions of Azerbaijan for the bioconversion suitability of xylotroph species and the synthesis of proteolytic enzymes was set as a goal.

In order to achieve the set goal, first, 107 strains of xylotroph species of basidiomycetes distributed in natural and artificial forests located in different areas of Azerbaijan were extracted from the fruit bodies, and the species composition was determined and included in the collection. The strains included in the collection belong to a total of 25 species of xylotrophic macromycetes and they are characterized by different indicators (diversity according to ecotrophic relationships, rot color, hyphal systems, life time and weight of FB, as well as growth factor). These differences are based on the noticeable diferences in their enzyme system, including proteolytic activity, which have been confirmed in the course of research.

Thus, the proteolytic activity of strains belonging to fungi such *as Armillaria mellea, Bjerkandera adusta , Fomitopsis pimicola, Fomes fomentarius, Ganoderma lucidum, Laetiporus sulphureus, Inonotus hispidus,* which are not caharacterized by such different indicators according to the growth factor, was higher than others, and it is interesting that these fungi not all of them belong to saprotrophs in terms of ecotrophic relationships, i.e. they are either biotrophs (*Amillaria mellea, Fomes fomentarius* and *Inonotus hispitus*), or their biotrophy or saprotrophy is not true.

In true saprotrophs *(Polyporus varius, Trametes hirsute, T.versicolor, T.zonata* and *Schizophyllum commune* the quantitative index of proteolytic activity is characterized by a relatively low index. First of all, this fact allows us to note that the proteolytic enzyme system is related to the pathogenic activities of fungi, that is, the high proteolytic activity enables the pathogenic activity of its producer increase. this fact was "*confirmed in the research*

conducted with micromycetes"[17] and in the example of macromycetes spread in Azerbaijan, this fact was confirmed precisely as a result of these studies.

On the other hand, according to the obtained results, fungi such as *B.adusta* P-40, *C.unicolor* M-3, *P.ostreatus* P-118, *T-hirsute* M-5 and *T.versicolor* D-13, which don't have high activity of proteolytic enzymes, are also useful where was also clarified in the course of research and it was justified that their use in both microbiological and enzymological conversion of lignocellulose – containing plant substrates is promising.

As a preliminary result of the screening of the studied xylotrophic macromycete strains, fungi such as *G.applanatum* and *I.hispidus* were selected for the next stage as active producers. İn the next stage, it was considered appropriate to use the strain of B.adusta fungus for the comparitive study of the proteolytic enzyme system specific to fungi.

In the second stage of research, the synthesis of proteolytic enzymes in the selected strains, its regulation depending on the carbon and nitrogen sources, and the determination of the type of proteases synthesized by fungi were clarified. İt became clear that the synthesis of proteases in all 3 fungi is constitutively synthesized according to the classical approach (Jakob – Mono), but according to the unitary theory, this process is realized inductively. On the other hand, it became clear that the quantitative indicators of the part of proteases secreted outside the cell vary depending on the carbon source, and as the degree of polymerization of carbon sources, namely saccharides increases, the quantitative indicators of the secreted enzyme also increase.

These data can also be evaluated as information that sends light on the synthesis of proteolytic enzymes depending on the carbon source. Thus, during enzyme production "*exogenous*

[17] León Rodríguez K., Higuera B.L. and Martínez S.T.Induction of proteases secreted by Fusarium oxysporum f. sp. dianthi in the presence of carnation root cell walls. biochemical characterization of a serine protease//Journal of Plant Pathology, 2017,v.99, N3, p.609-617.

enzymes are considered more important from a biotechnological point of view"[1818].

İt is known that proteolytic enzymes are caharacterized according to the structure of their active centers and the acidity of the environment where the effect occurs. İt became clear from the conducted studies that the *proteases* synthesized by fungi are characterized by diversity in this respect, and this diversity changes from symbiotrophy to biotrophy.True, although there is no clearly expressed dependence between the ecotrophic distribution of fungi and the type of proteases, the relative amount of individual types of proteases in polytrophs and true biotrophs is more widely followed. These data are important for determining the field of use proteases synthesized by fungi.

On the other hand, as mentioned, the 3 types of fungi selected as active producers in the studies also differ from each other in terms of ecotrophic relationships, that is, the biological state of the source of organic matter necessary for life activity. Thus, I.hispidus has characteristics typical of true biotrophs, G.applanatum polytrophs, and B.adusta saprotrophs. İn our opinion, this ecotrophic difference in fungi is one of the main reasons for the formation of specific features observed in the synthesis of proteolytic enzymes of fungi. İf we approach the results considered fromthis point of view, it is clear that proteolytic fungi are more powerful and diverse in fungi belonging to polytrophs, and this diversity is characterized by relatively low indicators in both biotrophs and saprotrophs. This is due to the fact that polytrophs have evolved an enzyme system that catalyzes the degradation and transformation of organic substances in two major states in order to obtain the organic matter needed for nutrition from a wide range of sources – both living and non-living-depending on their biological state Both Saprotrophs and biotrophs have formed an enzyme

[18] Giehl A., dos Santos A.A., Cadamuro R.D. et al. Biochemical and Biotechnological Insights into Fungus-Plant Interactions for Enhanced Sustainable Agricultural and Industrial Processes.//Plants, 2023. 12(14):2688. https://doi.org/10.3390/plants12142688.

system corresponding to the source from which they receive organic matter.

These data obtained in the research are expressed in the form of the following 5 results;

RESULTS

1. 107 strains of 25 species of xylotrophic macromycetes collected from different areas of natural artificial forests located in Great Caucasus, Kura- Araz plain and Talish mountains of Azerbaijan were cultured and a collection are characterized by diversity according to the color of the decay they cause under natural conditions, hyhphal systems, the shape of the fruit body they form under natural conditions, the duration of life, weight and the environmental parameters of the synthesis process[3, 11, 14].

2. İt was determined that, in terms of ecotrophic relationships, the proteolytic activity of fungi belonging to true bioptrophs and polytrophs is higher than those belonging to true saprotrophs, but the latter are characterized by higher lignolytic and cellulolytic activity. This made it possible to note that it is appropriate to use polytrophs and biotrophs in the conversion of proteolytic and saprotrophs in the conversion of lignocellulosic substrats[5-6, 8, 10-11].

3. Proteases syhnthesized by xylotrophic macromycetes differ according to the structure of their catalytic centers, and serine-, cysteine- and metallo-proteases are found among the proteases they synthesize, but their ratio varies depending on the fungi. Although there is no clearly expressed dependence between the ecotrophic distribution of fungi and the type of proteases, the relative amount of different types of proteases in polytheophas and true biotrophs is more widely followed[8, 10, 14].

4. İn xylotrophic macromycetes selected as active producers of proteolytic enzymes, the synthesis ofproteolytic enzymes takes place in a constitutive way according to Jakob-Mono theory, and inductive way according to the unitary theory and the complexation of the composition of the carbon source in the direction of mono-, di- and poly-saccharides determines the increase of the specific gravity of the part of the synthesized enzyme secreted outside the cell up to 12-18%[2, 4, 7, 9].

5. During the addition of the aqueous extract taken from the substrate where it is settled to the medium, more biomass accumulation occurs in the Inonotus hispidus fungi, which also causes an icrease in the activity of proteolytic enzymes. On the other hand, during the maintenance of the working culture, the addition of the material taken from the host plant and powdered to the nutrient medium also leads to positive results, which is due to the fact that the fungus acquires a certain adaptation sign resulting from the biotrophic lifestyle. This also allows the fungus to absorb both the ammonia and nitrate forms of the mineral nitrogen source equality[1, 2, 12-13].

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