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ABSTRACT

Of the dissertation for the degree of Doctor of Philosopy

ASSESSMENT OF THE BIOTECHNOLOGICAL POTENTIAL OF MICROMYCETES ISOLATED FROM EXTREME ENVIRONMENTS BASED ON THEIR ANTIMICROBIAL AND ENZYMATIC PROPERTIES

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INTRODUCTION

Relevance of the topic and the degree of its development. The growth of the world's population, occurring within a relatively stable area of the Earth's surface, gives rise to increasingly evident difficulties in meeting the demand for energy, food, and raw materials for various industries using existing resources and opportunities. "*It is worth recalling just one fact: according to some forecasts, between 2010 and 2050, the global demand for food products will increase by 45–56%, while the proportion of the population at risk of hunger will change from 8% to as high as 91%* [1]"

Therefore, addressing these challenges, managing the problems that will inevitably emerge, and ensuring the sustainable development of society constitute urgent and essential tasks before modern science, primarily biology and its applied field—biotechnology.

Traditionally, plants and animals have played the leading role in meeting humanity's demand for food, energy, and raw materials for various industries, and they still retain this status today. However, since ancient times, microorganisms have also contributed to human nutrition; the earliest processes that may be considered biotechnological today (such as bread baking, winemaking, and others) were based on the participation of fungi. Although plants and animals remained the main sources of human livelihood for a long time, advances in science and technology have significantly expanded the possibilities of utilizing microorganisms, and this process continues to develop.

"The reason is that metabolites produced as a result of microbial activity are favorable and cost-effective for meeting

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[1] Van Dijk, M., Morley, T., Rau, M.L. et al. A meta-analysis of projected global food demand and population at risk of hunger for the period 2010–2050. // Nat Food, 2021, v.2, p.494–501.

human needs for food, energy, and other purposes."[2,3] Among the metabolites of microorganisms are "*enzymes, polysaccharides, antibiotics, vitamins, organic acids, and other substances with biological, including pharmacological, activity*"[4]. Their production is advantageous "*from ecological, economic, and technological perspectives.*"[5] Microorganisms differ positively from plants and animals in their growth rate, and during growth, they can transform diverse plant wastes (today considered industrial waste) into products rich in proteins, sugars, fats, and other valuable compounds. Furthermore, the cultivation of microorganisms under artificially created conditions requires much simpler and cheaper technologies compared to plants and animals. Microorganisms also yield higher productivity per unit area compared to plants and animals. All of these factors make microorganisms—especially bacteria and fungi—an object of growing scientific and industrial interest, leading to the expansion of both the range and the volume of products derived from them.

Nevertheless, despite their importance, the utilization of microorganisms to meet human nutritional needs has not yet reached the desired level. Although the number of microbial species on Earth *is estimated in the hundreds of thousands or even millions*[6], fewer than 1% are exploited on an industrial scale. The main reason for this is the limited and incomplete knowledge regarding the potential of most species as producers of biologically active substances (BAS) for food, feed, medical, and technical purposes.

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[2] Prajapati N., Patel J., Singh S. et al. Postbiotic production: harnessing the power of microbial metabolites for health applications// Front. Microbiol., 2023, 14:1306192. doi: 10.3389/fmicb.2023.1306192

[3] Singh, R., Kumar, M., Mittal, A. et al. Microbial metabolites in nutrition, healthcare and agriculture. //3 Biotech, 2017, **7**, 15. https://doi.org/10.1007/s13205-016-0586-4

[4] Hashem, A.H., Attia, M.S., Kandil, E.K. et al. Bioactive compounds and biomedical applications of endophytic fungi: a recent review//Microb Cell Fact, 2023,22, 107. https://doi.org/10.1186/s12934-023-02118-x

[5] Muradov, P.Z. Bitki tullantılarının biokonversiyasının əsasları. –Bakı: "Elm" nəşriyyatı, 2003, 114s.

[6] Wiens, J.J. How many species are there on Earth? Progress and problems//PLoS Biol., 2023, 20;21(11): e3002388. doi: 10.1371/journal.pbio.3002388.

Another notable trend observed in the analysis of scientific literature is that most research centers worldwide focus on different strains of the same fungal species. For example, in many countries (USA, Russia, China, India), "*the fungus Ganoderma lucidum is extensively studied as a producer of BAS"*[7,8]. This approach is due not only to the perceived promise of such fungi but also to the influence of natural soil-climatic conditions and local flora on the biosynthetic capacity of the strains, making it possible to discover more active strains in specific environments.

In parallel with the above-mentioned issues, the modern era is also characterized by the emergence of global problems resulting from the intensifying anthropogenic pressure on the environment. These include climate change, the deterioration of ecological conditions, loss of biodiversity, soil salinization, desertification, and others. Such phenomena further aggravate an already fragile situation by altering the character and direction of relationships within existing ecosystems. Addressing these challenges requires the study of processes occurring in ecosystems exposed to extreme or stress conditions.

On the other hand, the investigation of living organisms inhabiting such environments—primarily microorganisms—is also of practical interest. "*Microorganisms that inhabit such biotopes, generally referred to as extremophiles"*[9], are sources of biologically active substances (BAS), especially enzymes, that maintain stability over a broad range of temperatures and pH values. Furthermore, microorganisms capable of surviving in environments heavily polluted with various pollutants hold promise for the bioremediation of contaminated areas. Extremophiles are also valuable as reservoirs

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[7] Kachrimanidou, V., Papadaki, A., Papapostolou, H. et al. *Ganoderma lucidum* Mycelia Mass and Bioactive Compounds Production through Grape Pomace and Cheese Whey Valorization//Molecules, 2023, 28(17), 6331. https://doi.org/10.3390/molecules28176331

[8] Ahmad, M.F., Wahab, S., Ahmad, F.A. et al. Ganoderma lucidum: a potential pleiotropic approach of ganoderic acids in health reinforcement and factors influencing their production//Fungal Biol. Rev., 2022, v.39, -p.100–125. doi: 10.1016/j.fbr.2021.12.003

[9] Durvasula, R. V. Extremophiles: From biology to biotechnology / R. V. Durvasula, D. V. S. Rao. – CRC Press, 2018. – 437 p.

of genes enabling the creation of novel recombinant super-producers and for obtaining more effective agents for biological control. All these factors explain the increasing interest in extremophilic microorganisms.

As part of the global biosphere, the Republic of Azerbaijan also harbors ecosystems subjected to extreme conditions. Examples include oil-contaminated soils, saline lands, and thermal water sources. Although certain studies have been conducted in these environments, the existing results are insufficient to fully clarify the processes occurring there or to evaluate the biosynthetic potential of the resident microorganisms, particularly micromycetes.

Purpose and objectives of the research.

Accordingly, the aim of the present study is the isolation of micromycetes from extreme habitats in Azerbaijan and their evaluation in terms of antibiotic and enzymatic activities.

To achieve this aim, the following objectives were defined as necessary:

1.To isolate micromycetes (and some macromycetes) from various extreme biotopes of Azerbaijan and to establish a collection of strains;

2.To evaluate the enzymatic (hydrolase and oxidase) activity and antibiotic properties of the isolated pure cultures;

3.To determine the taxonomic affiliation of selected active producers and to assess their biotechnological potential;

4.To identify the biological effects of endogenous and exogenous metabolites synthesized by promising producers and to evaluate their potential applications.

Research methods.

To achieve the stated aim and objectives, the study employed methodologies commonly used in mycology, microbiology, and biotechnology. The precision of instruments and equipment, the purity of reagents, the reproducibility of experiments (carried out at least 4–6 times), and the statistical processing of data ensured the reliability of the results.

Provisions for Defense

- Biotopes with extreme environmental conditions can serve as habitats for both macromycetes and micromycetes.

- The antimicrobial and enzymatic activities of fungi isolated from biotopes with different ecological characteristics vary depending on the environmental conditions.
- The differential influence of carbon sources on the fungi isolated during the study determines the diversity in the nature of enzyme synthesis.
- While optimization of culture conditions generally leads to increased activity of all enzymes, the degree of polymerization of carbon sources specifically enhances the extracellular secretion of cellulases synthesized by fungi.

Scientific Novelty of the Research

As a result of the conducted studies, fungi distributed in various extreme ecosystems of Azerbaijan were evaluated for their antimicrobial and enzymatic activities. For this purpose, samples were collected from oil-contaminated areas, high-salinity zones, thermal water sources, and highly humid subtropical forests. Based on these, a collection of 76 fungal strains was established, comprising 64 micromycetes and 12 macromycetes.

It was determined that 75% of the isolated micromycetes are mesophilic in terms of temperature preferences, while 25% display thermotolerant characteristics, maintaining viability at temperatures above 50°C. Among the micromycetes, between 25.0% and 73.4% exhibited both hydrolase and oxidase activity; for macromycetes, this figure ranged from 25.0% to 100%.

It was established that the fungi *Aspergillus candidus, Chaetomium thermophile, Penicillium resticulosum, Humicola lanuginosa, and Mucor hiemalis* are producers of enzymes with hydrolytic activity. *Bjerkandera adusta* was shown to possess a balanced enzymatic system with both hydrolase and oxidase activities, *Trametes hirsuta* demonstrated high oxidase activity, while *Trichoderma viride* was identified as an active producer of endoglucanase. These characteristics highlight the potential of these fungi as valuable sources for enzyme production.

Among the isolated strains, 53.1% of micromycetes and 58.3% of macromycetes exhibited antimicrobial activity against the test cultures *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Candida albicans.* The highest antimicrobial activity was observed in *Aspergillus flavus, Chaetomium globosum, Penicillium chrysogenum, P. citrinum, Trametes hirsuta, and Trichoderma viride.*

Optimization of cultivation conditions aimed at enhancing fermentative activity resulted in a 15–20% increase in enzyme yield, whereas optimization for antibiotic activity led to an 11–17% improvement.

Analysis of enzymatic regulation revealed that protease synthesis in all selected active producers, as well as the synthesis of other hydrolases in micromycetes, occurred constitutively. In contrast, cellulase synthesis in *Trichoderma viride* and macromycetes was induced and subject to catabolite repression. Oxidase synthesis in macromycetes was determined to be constitutive according to the classical Jacob–Monod model, but inductive in line with the unitary theory.

A weak but consistent positive correlation was identified between the degree of polymerization of carbon sources and the extracellular secretion of synthesized enzymes. Moreover, nitrate proved to be the most favorable nitrogen source for micromycetes, while ammonium was more advantageous for macromycetes.

Theoretical and Practical Significance of the Research

The results obtained serve as factual material that contributes to expanding the information on the antimicrobial and enzymatic activities of fungi distributed under extreme conditions.

During the course of the research, strains isolated from extreme environments and selected as active producers have been identified as potentially favorable for the future production of antimicrobial agents, as well as enzymatic preparations with broad-spectrum hydrolase and oxidase activities. This indicates that their application could lead to the acquisition of effective results.

The findings concerning the optimization of cultivation conditions demonstrate that fungi can also be utilized in the production of exogenous enzymes with high activity, thereby creating practical opportunities for their industrial application.

Publications, Dissertation Approval, and Implementation

A total of 11 scientific works related to the results of the dissertation have been published, including 6 scientific articles. The materials of the dissertation were presented at the following scientific events: the 5th International Scientific-Practical Conference "Current Problems of Biological and Chemical Ecology" (Moscow, 2016), the Republican Scientific Conference "Modern Problems of Biology" (Sumgait, 2018), the Scientific-Practical Conference "Current Issues of Modern Biology" (Baku, 2019), the 12th International Scientific-Practical Conference "Current Scientific Research" (Penza, Russian Federation, 2023), and the International Mycological Forum (Moscow, 2025).

Institution Where the Dissertation Was Carried Out

The dissertation was conducted at the Laboratory of Microbiological Biotechnology of the Institute of Microbiology of the Ministry of Science and Education of the Republic of Azerbaijan.

Structure and Scope of the Dissertation

The dissertation has been prepared in accordance with generally accepted principles and consists of an introduction, four chapters, a final analysis of the research, conclusions, and lists of references and abbreviations. The total volume of the dissertation amounts to 217,500 characters.

CHAPTER I

GENERAL CHARACTERISTICS OF EXTREME CONDITIONS IN TERMS OF ABIOTIC AND BIOTIC FACTORS

Section 1.1 provides an overview of the concept of extreme environments and their general characteristics. Section 1.2 presents the general characteristics of the distribution of micromycetes in such environments, while Section 1.3 analyzes the extreme cenoses of the Republic of Azerbaijan and the level of study of their fungal biota, thereby clarifying the research status of the problem in accordance with the objectives of the study.

CHAPTER II
MATERIALS AND METHODS OF RESEARCH

2.1. Research Areas and Their General Characteristics

Samples for the study were collected from oil-contaminated and highly saline soils of the Absheron Peninsula, subtropical forests (Lankaran – Astara economic region), and thermal water sources of the Greater Caucasus. A total of about 500 samples were collected from these ecosystems, and they were used for the isolation of pure fungal cultures. For sampling, the standard "*methods and approaches*"[10] employed in similar studies were applied.

2.2. Methods and Approaches Used for Analysis

At least 100 samples were taken from each selected area, documented in situ, and transported to the laboratory within a short period (2–6 hours) for further analysis.

To isolate fungi, 10% suspensions were prepared from soil samples in sterile water and incubated at room temperature for 2 hours. The suspensions were then either directly or after dilution (10× and 100×) plated onto nutrient media (malt extract agar – MEA, and Sabouraud agar). After 2–3 days, colonies formed on the media were distinguished by their morphological features, transferred to fresh media, and subcultured until homogeneous biomass was obtained. Purity was verified under a microscope (OMAX 40X–2500X). Species identification was carried out based on classical mycological approaches using standard "*determinants.*"[11]

For macromycetes, basidiomata collected from trees in humid subtropical forests were isolated, cultured (MEA, 2–40B), and their

[10] Maheshwari, R. Fungi: Experimental Methods In Biology, 2th Edition. CRC Pres,2016, 358p

[11] Kirk, P. M. et al. Dictionary of the fungi, 10th edn. CABI publishing – Wallingford (UK), -2008, -600 p.

species composition determined using "*standard methods*."[12,13,14] To assess enzymatic activity, liquid Czapek medium was used for micromycetes and glucose–peptone medium for macromycetes, with submerged cultivation applied. Enzymatic activity was measured for endoglucanase (substrate: 1% Na-carboxymethylcellulose), xylanase (xylan), amylase (soluble starch), protease (Na-caseinate), lipase (olive oil), pectinase (apple pectin), laccase (hydroquinone), and peroxidase (hydroquinone + hydrogen peroxide) "*appropriate methods and approachs.*"[15,16] Antibiotic activity was tested against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Candida albicans,* provided by the Laboratory of Microbiological Biotechnology of the Institute of Microbiology of the Ministry of Science and Education of Azerbaijan. Test cultures were exposed to endogenous and exogenous metabolites obtained from the fungi for 30 and 60 minutes before inoculation into nutrient media. Growth inhibition was assessed, and the antibiotic activity of the most active cultures was additionally confirmed using the "*disk diffusion method.*"[17]

The biotechnological potential of the selected active producers was determined according to "*current principles and standards*"[18,19,20] in the field. All experiments were conducted with

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[12] Maheshwari, R. Fungi: Experimental Methods In Biology, 2th Edition. CRC Pres, 2016, 358p

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

[14] Змитрович, И. В. Определитель грибов России / И. В. Змитрович. – М. – СПб.: Товарищество научных изданий КМК, 2008. – 278 с.

[15] Vermelho A.B., Couri S. Methods to Determine Enzymatic Activity. Bentham Science Publishers, 2013. https://doi.org/10.2174/97816080530011130101

[16] Полыгалина Г.В., Чередниченко В., Римарева Л.В. Определение активности ферментов. Справочник. –Москва: ДеЛи, 2004, 376с.

[17] Определение чувствительности микроорганизмов к антибактериальным препаратам: Методические указания. — М.:ФЦ Госсанэпиднадзора Минздрава России, 2004.— 91 с

[18] Bentahar S., Abada R., Ykhlef N. Biotechnology: definitions, types and main applications//YMER Digital, 2023, 22(1), iss.4, -p.563-575

[19] https://store.astm.org/products-services/standards-and-publications/standards/biotechnology-standards.html

[20] Rashidi L. Standards for Modern Biotechnology: Navigating Innovation and Responsibility//Advances in the standards & applied sciences 2025; 1 (3), -p.1-3

at least four replicates. The results were "*statistically*"[21] processed using standard deviation analysis, and only data meeting the criterion $S/\chi o \leq 0.05$ were included in the dissertation.

CHAPTER III
EVALUATION OF FERMENTATIVE ACTIVITY AND ANTIBIOTIC PROPERTIES OF FUNGI ISOLATED FROM EXTREME CONDITIONS

3.1. General Characteristics of Strains Isolated from Extreme Environments

Analysis of samples taken from extreme habitats in the Republic of Azerbaijan confirmed the isolation of 76 pure fungal strains. Their distribution by origin is presented in Table 3.1. The.

Table 3.1

Distribution of fungal strains by sampling sites

№	Sampling site	Number of strains isolated	Share in total (%)
1	Oil-contaminated soils (OS)	21	27.6
2	Lake Masazir (LM)	17	22.4
3	Thermal springs (TS)	9	11.8
4	Subtropical forests (SF)	29	38.2
Total		76	100

data indicate that strains belonging to a given species were unevenly distributed across different environments, which is associated with differences in the ecological conditions of each biotope as well as the chemical composition and concentration of available nutrients

Approximately 16% of the pure cultures obtained (12 strains)

[21] Зверев, А.А., Зефиров, Т.Л. Статистические методы в биологии: учебно-методическое пособие. -Казань, КФУ, -2013. - 42 с.

belonged to xylotrophic macromycetes, all isolated from the forests of the southern region. Their inclusion aimed at comparative analysis with micromycetes. It was found that among the isolated strains there were fungi capable of efficiently carrying out the bioconversion of plant residues (PR), and their activity in this respect was significantly higher than that of micromycetes, which is associated with the presence of more powerful enzymatic systems. In some cases, cultures were isolated directly from the collected samples; in others, samples were incubated at 50 °C for 24 hours prior to isolation. Characterization of the obtained strains revealed that the majority belonged to mesophiles (Table 3.2).

Table 3.2

Temperature characteristics of micromycete strains isolated

№	Sampling site	| Number of strains		Share in total (%)	
		MF	TT	MF	TT
1	OS	16	5	33,3	31,3
2	LM	13	4	27,1	25,0
3	TS	6	3	12,5	18,7
4	SF	13	4	27,1	25,0
Total		48	16	100	100

As seen, among the 64 micromycete strains isolated, 75% were mesophilic (MF), while 25% belonged to thermotolerant (TT) types. Incubation of samples at 75 °C for 24 hours showed that no fungal structural elements (spores, hyphal fragments, or reproductive structures) could retain viability, confirming the absence of true thermophilic fungi among the isolates.

3.2. Screening of Fungi Isolated from Extreme Environments for Fermentative Activity and Selection of Active Producers

Studies on the fermentative activity of fungal cultures isolated from extreme environments showed that while most produced one or more enzymes, the proportion of strains exhibiting activity varied depending on the enzyme (Table 3.3).

Table 3.3

Preliminary evaluation of enzymatic activity of isolated micromycetes

№	Enzymes	Number of strains isolated showing activity							
		OS		LM		TS		SF	
		I	II	I	II	I	II	I	II
1	Endogluca-nase	16/11	5/3	13/9	4/2	6/3	3/2	13/7	4/2
2	Protease	16/13	5/4	13/10	4/3	6/3	3/2	13/9	4/3
3	Pectinase	16/10	5/3	13/8	4/2	6/2	3/1	13/8	4/1
4	Amylase	16/9	5/3	13/9	4/2	6/3	3/1	13/7	4/2
5	Xylanase	16/7	5/2	13/6	4/2	6/2	3/1	13/6	4/1
6	Lipase	16/8	5/3	13/7	4/2	6/3	3/2	13/8	4/2
7	Laccase	16/4	5/2	13/4	4/1	6/2	3/1	13/4	4/1
8	Peroxidase	16/3	5/1	13/4	4/1	6/2	3/1	13/3	4/1

The results showed that the highest number of strains exhibited proteolytic activity, while the lowest was recorded for oxidase activity. Specifically, out of 64 micromycete strains, 44 displayed proteolytic activity, whereas only 16 produced peroxidase, meaning the former were 2.75 times more numerous. Similar quantitative differences were observed among other enzyme activities, as well as between micromycetes and macromycetes.

In general, among the 64 micromycete and 12 macromycete strains isolated, enzymatic activity levels were distributed as presented in Table 3.4.

Table 3.4.
General Characteristics of Fungal Strains Based on Their Fermentative Activity

№	Enzymes	Number of Strains Isolated and Active (micromycetes/macromycetes)		Percentage of Strains in Total (%)	
		Micromycetes	Macromycetes	Micromycetes	Macromycetes
1	Endoglucanase	64/39	12/12	60,9	100
2	Protease	64/47	12/10	73,4	83,3
3	Pectinase	64/35	12/ 8	54,7	66,7
4	Amylase	64/36	12/7	56,3	58,3
5	Xylanase	64/27	12/12	42,2	100
6	Lipase	64/35	12/3	54,7	25,0
7	Laccase	64/19	12/9	29,7	75,0
8	Peroxidase	64/16	12/9	25,0	75,0

As shown in the table, the fermentative system of macromycetes is stronger than that of micromycetes, which is evidenced by the activity of oxidases, endoglucanase, and xylanase. All macromycetes exhibit activity for endoglucanase and xylanase, and 3/4 of them show activity for laccase and peroxidase. In comparison, the number of active strains of micromycetes is three times fewer than that of macromycetes. Furthermore, for endoglucanase and xylanase activity, the macromycetes again demonstrate superior performance, as all macromycetes exhibit activity for both enzymes, while micromycetes show activity for endoglucanase and xylanase in only 42.2% to 60.9% of strains.

It is important to note that strains belonging to both micromycetes and macromycetes differ not only in their ability to exhibit activity for certain enzymes but also in the level of this activity. For example, among the 64 strains observed, 39 strains exhibited endoglucanase activity, with activity levels ranging from 0.08 to 1.4 BV/ml, meaning the maximal and minimal values differed by a factor of 17.5. Similar differences in activity levels were observed for xylanase, protease, amylase, and lipase, with variances ranging from 10.5 to 23.7 times in micromycetes. Similar differences were also noted for macromycetes, where the activity of laccase and peroxidase varied by factors of 1.5–3 and 1.2–2.3, respectively.

As discussed, the fermentative activity of macromycetes is considered stronger than that of micromycetes, which is likely related to their more balanced fermentation system that catalyzes the degradation of cellulose, lignin, and hemicellulose — the key components of plant biomass and, after their use, of solid waste materials.

Thus, based on preliminary evaluation, it was found that 25.0% to 73.4% of micromycete strains exhibit activity for one or more of these enzymes. Based on these findings, the selection of the most active strains for the next stage is intended to enable the effective utilization (bioconversion) of large quantities of plant biomass and waste materials. This involves catalyzing the degradation of polymers such as cellulose, lignin, hemicellulose, etc., or optimizing the synthesis of enzymes with the highest activity levels for targeted applications.

Based on the above, 8 strains were selected for further study. Among these, 2 are macromycetes and 6 are micromycetes. The species composition and the criteria for selecting these strains were determined through further identification and clarification of their type and functionality (see Table 3.5).

Table 3.5

General Characteristics of Fungal Strains Selected as Active Producers

№	Species Name	Sampling Location	Criteria for Selection
1	*Aspergillus candidus*	LM	Hydrolases
2	*Chaetomium thermophile*	TS	Hydrolases
3	*Bjerkandera adusta*	SF	Balanced enzyme system
4	*Humicola lanuginosa*	TS	Hydrolases
5	*Penicillium resticulosum*	LM	Hydrolases
6	*Mucor hiemalis*	OS	Hydrolases
7	*Trametes hirsuta*	SF	Hydrolases
8	*Trichoderma viride*	SF	Hydrolases

It should be noted that the activity profiles of fungi varied for different biotopes. For example, micromycetes isolated from thermal waters, Masazir Lake, and oil-contaminated soils showed lower endoglucanase activity than those isolated from subtropical forests. This difference may be attributed to the different adaptive responses of the fungi to the degradation processes occurring in those ecosystems and their distinct reactions to the extreme factors that lead to these conditions.

3.4. Screening of Fungi Isolated from Extreme Environments Based on Antibiotic Activity and Selection of Active Producers

In the studies on the antimicrobial activity of 76 fungal strains, it was found that among the strains, there were both those exhibiting antibiotic activity and those that did not. The results are presented in

Table 3.6. As shown, both micromycetes and macromycetes are represented among the strains with antibiotic activity, and the proportion of active strains is slightly higher in macromycetes.

It is important to note, based on the data in Table 3.6, that the antibiotic activity of the isolated strains shows a characteristic trend. Specifically, the antibacterial and antifungal activities vary among the strains. In some cases, strains exhibit higher antibacterial activity compared to antifungal activity, while in others, the opposite is observed. In some strains, both activities are almost at the same level. It should be noted that fungal strains exhibiting antibacterial activity also tend to have antifungal activity, though with varying intensities.

Table 3.6

Characteristics of Isolated Strains Based on Antibiotic Activity

No.	Sampling Location	Number of Strains Isolated and Active		Percentage of Strains in Total (%)	
		Micromy-cetes	Macro-mycetes	Micro-mycetes	Macro-mycetes
1	OS	21/14	0	66,7	0
2	LM	17/9		52,9	
3	TS	9/4		44,4	
4	SF	17/7	12/6	41,2	58,3
Total		64/34	12/6	100/53,1	100/58,3

Based on the results of the screening for antibiotic activity, 6 strains were selected as active producers. The selection of these strains was based on their high antibacterial, antifungal, or both activities. For each group, two of the most active strains were selected. Upon determining the taxonomic affiliation of the selected strains, it was found that one (Trametes hirsuta) belongs to the macromycetes, while five others (Aspergillus flavus, Chaetomium globosum, Penicillium chrysogenium, P. citrinum, and Trichoderma viride) belong to the micromycetes.

It is worth noting that three types of antimicrobial activity are distinguished based on the size of the lysis zone. Specifically:

- Weak activity is characterized by a lysis zone diameter of less than 20 mm,
- Strong activity is characterized by a lysis zone diameter greater than 29 mm,
- Moderate activity is characterized by a lysis zone diameter between 20 mm and 29 mm.

When characterizing the strains according to this classification, it becomes clear that only the strain Trichoderma viride exhibits strong antimicrobial activity, with a lysis zone diameter ranging between 29–34 mm, depending on the test culture used.

Out of the strains selected for their fermentative activity and antibiotic properties, only two coincide: the strains from Trametes hirsuta and Trichoderma viride. Thus, the total number of species selected for their fermentative and antibiotic properties amounts to 12 (see Table 3.7).

3.5. Annotated List of Fungal Species Selected as Active Producers Based on Fermentative and Antibiotic Activity

Based on the screening results, 12 fungal species were selected as active producers due to their combined antimicrobial and fermentative activities. The annotations for these species include their systematic status, sampling location, substrate, and details of their fermentative and antibiotic activities. These details are provided based on both local and global research on the respective species.

Table 3.7

General Characteristics of Fungal Strains Selected as Active Producers

No.	Species Name	Sampling Location	Selection Criteria
1	*Aspergillus candidus*	LM	Hydrolases
2	*Chaetomium thermophile*	TS	Hydrolases
3	*Bjerkandera adusta*	SF	Balanced enzyme system
4	*Humicola lanuginosa*	TS	Hydrolases
5	*Penicillium resticulosum*	LM	Hydrolases
6	*Mucor hiemalis*	OS	Hydrolases
7	*Trametes hirsuta*	SF	Oxidoreductases and antimicrobial activity
8	*Trichoderma viride*	SF	Endoglucanase and antibacterial activity
9	*Penicillium chrysogenium*	TS	Antimicrobial
10	*Chaetomium globosum*	OS	Antifungal
11	*Penicillium citrinum*	TS	Antibacterial
12	*Aspergillus flavus*	LM	Antifungal

CHAPTER IV
SELECTION OF OPTIMAL GROWTH MEDIUM FOR PRODUCERS ISOLATED FROM EXTREME ENVIRONMENTS AND DETERMINATION OF THE IMPACT OF THE ENDOGENOUS AND EXOGENOUS METABOLITES THEY SYNTHESIZE

4.1. Optimization of the Medium for Strains Selected as Active Producers Based on Antibiotic and Fermentative Activities

While the synthesis of any metabolite in an organism is inherently linked to its genomic characteristics, it is also well established that environmental factors play a crucial role in the production of such metabolites. In light of this, the research conducted in this phase aimed to identify the optimal medium that facilitates maximal expression of fermentative activity for the strains selected as active producers. The primary parameters of the medium, including the sources of carbon and nitrogen, cultivation methods, temperature, pH, preparation techniques, and duration for inoculum cultivation, were all optimized.

The results indicated that, for some fungal strains, the parameters required to exhibit fermentative activity were consistent across all strains, while others varied (Table 4.1). Based on the optimization studies, the following key scientific and practical observations were made:

1. Proteolytic enzyme synthesis in both micromycetes and macromycetes occurs constitutively.
2. Except for Trichoderma viride, all micromycetes synthesize hydrolases constitutively.
3. In Trichoderma viride and macromycetes, cellulase synthesis occurs inductively and is regulated by catabolic repression.
4. The synthesis of oxidases in macromycetes follows the Jakob-Monod model for constitutive synthesis and is regulated by the unitary theory for inducible synthesis.
5. A direct relationship was found between the polymerization

degree of carbon sources and the proportion of extracellular activity, which is more distinctly observed in macromycetes.

6. The use of organic solvents (such as acetone) was found to be more effective for obtaining partially purified technical enzyme preparations from the active producer strains.

7. In macromycetes, the basal level of inducible enzyme synthesis is significantly higher when compared to micromycetes.

8. In micromycetes, nitrate nitrogen proved to be more effective, while ammonia nitrogen was more effective for macromycetes.

For the strains selected as active producers based on antibiotic activity, the conditions required to achieve maximal antibiotic production were also determined, characterized by both common and distinct parameters (Table 4.2). It was found that the carbon source concentration required for optimal antibiotic activity was lower in comparison to that required for maximal fermentative activity.

The optimization of conditions for both antibiotic and fermentative activities resulted in a notable increase in the yield of target metabolites, with a 15–20% increase in fermentative activity and an 11–17% increase in antibiotic activity.

In conclusion, the studies provided clarification on the specific applications and characteristics of the 12 selected strains as active producers. Furthermore, it was identified that certain strains lacked detailed data regarding their potential uses (Table 4.3). Based on the acquired findings, it was concluded that utilizing these strains in agricultural sectors presents more significant advantages.

COMPREHENSIVE ANALYSIS OF THE RESEARCH FINDINGS

The intensification of human impact on the environment, which has become a prominent feature of modern times, has led to the significant reduction of natural habitats, replaced increasingly by anthropogenically transformed environments. As a result, the formation of stressed biotopes is inevitable, where the parameters

Table 4.1

Optimized Growth Medium Parameters for Enzyme Synthesis in Fungal Strains

№	Species Name	Source and Quantity (g/l)		Cultivation		Initial pH	Inoculum Preparation Method and Duration (days)	Cultivation Duration (hours)
		C	N	method	T^0C			
1	*A.candidus*	Sucrose (25)	$NaNO_3$ (2,0)	LPF, DC	29±1	6,2	SPF, 7 days, Spor Suspension	96±2
2	*P.resticulosum*		$NaNO_3$(1,9)					
3	*B.adusta*	Wheat Bran (15)	Pepton (2,9), NH_4NO_3(1,5)		27±1	5,9	LQPM, DC, 5 days	110±3
4	*T.hirsuta*		Pepton (3,0), NH_4NO_3(1,4)		28±1			
5	*Ch.thermophile*	Qlucose 20	$NaNO_3$ 2.0±0.1		32±1	6.2	SPF, 7 days, Spor Suspension	96±4
6	*H.lanuginosa*	Sucrose (25)			30±1			
7	*M.hiemalis*	Wheat Bra			30±1			
8	*Trichoderma viride*	n (20)			28±1			

Table 4.2

Optimized Medium Parameters for Fungal Strains Selected as Active Producers Based on Antibiotic Activity

<table>
<tr><th rowspan="2">№</th><th rowspan="2">Species Name</th><th colspan="2">Source and Quantity (g/l)</th><th colspan="2">Cultivation Method</th><th rowspan="2">Initial pH</th><th rowspan="2">Inoculum Preparation Method and Duration (days)</th><th rowspan="2">Cultivation Duration (hours</th></tr>
<tr><th>C</th><th>N</th><th>üsulu</th><th>T0C</th></tr>
<tr><td>1</td><td>Trametes hirsuta</td><td>Wheat Bran, 14</td><td>Pepton (2,8), NH_4NO_3 (1,4)</td><td rowspan="6">LPF, DC</td><td>28±1</td><td>5,9</td><td>LQPM, DC, 5 days</td><td>113</td></tr>
<tr><td>2</td><td>Penicillium chrysogenium</td><td>Sucrose, 22</td><td rowspan="5">$NaNO_3$ (1,7±0,2)</td><td rowspan="5">29±1</td><td rowspan="5">6,2</td><td rowspan="5">SPF, 7 days, Spor Suspension</td><td rowspan="5">95±5</td></tr>
<tr><td>3</td><td>Trichoderma viride</td><td>Wheat Bran, 18</td></tr>
<tr><td>4</td><td>Chaetomium globosum</td><td>Sucrose, 23</td></tr>
<tr><td>5</td><td>Penicillium citrinum</td><td rowspan="2">Sucrose, 22</td></tr>
<tr><td>6</td><td>Aspergillus flavus</td></tr>
</table>

Table 4.3

Potential Applications of Active Producer Fungal Strains

№	Species Name	Potential Use	Purpose and Form
1	*Aspergillus candidus*	No data available	Production of hydrolytic enzyme preparations
2	*Chaetomium thermophile*	Food industry	Production of hydrolytic enzymes with high thermostability
3	*Bjerkandera adusta*	Agricultural	Bioconversion of waste, live culture, and SPF
4	*Humicola lanuginosa*	Agricultural	Compost production, live culture
5	*Penicillium resticulosum*	No data available	Preparation of enzyme preparations with hydrolytic activity
6	*Mucor hiemalis*	Agricultural and Medical	Compost production, fibrinolytic enzyme production
7	*Trametes hirsuta*	Agricultural and Food industry	Bioconversion of waste, animal feed additives, live culture, dry biomass, SPF
8	*Trichoderma viride*	Agricultural	Biological control, hydrolysis, live culture, and SPF
9	*Penicillium chrysogenium*	Agricultural and Medical	Production of antibiotic preparations
10	*Chaetomium globosum*	Veterinary	
11	*Penicillium citrinum*	Agricultural	
12	*Aspergillus flavus*	Agricultural	

necessary for the survival and proper functioning of organisms deviate significantly from the normal range. These conditions are referred to as extreme environments, and the organisms capable of adapting to such conditions are termed extremophiles.

Extreme environments, characterized by the combination of "*physical, chemical, and biological*"[22] factors, vary based on the degree to which these factors deviate from normal ranges, which ultimately defines the extremity of the environment. The resulting conditions, either singularly or in combination, influence the biological activities of the organisms inhabiting them. The biota of each extreme environment often exhibits specificity, which can be elucidated through experimental research.

In Azerbaijan, various ecosystems subject to extreme conditions, such as salt-affected lands and petroleum-contaminated water and soils, along with thermal springs, mud volcanoes, and humid subtropical forests, provide examples of extreme environments where microorganisms, including fungi, thrive. Despite this, existing research has not sufficiently assessed the biotechnological potential of microscopic fungi from these extreme conditions. Therefore, this study aimed to evaluate the fermentative and antibiotic activities of fungal strains isolated from extreme environments and establish a collection of strains with practical biotechnological potential.

Examples of such ecosystems include thermal waters, mud volcanoes, as well as subtropical forests characterized by high humidity. It is known that microorganisms, including fungi, are widespread in these habitats. However, the research conducted so far is insufficient to fully evaluate the biotechnological potential of microscopic fungi adapted to these extreme conditions. Therefore, this issue remains open for further investigation. Considering this, the present study aims to assess the biotechnological potential of microscopic fungi isolated from extreme habitats based on their fermentative and antibiotic activities, and to establish a collection of

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[22] https://app.studyraid.com/en/read/8997/255902/characteristics-of-extreme-environments

strains of practical interest.

To achieve this goal, key tasks were identified and addressed, and the obtained results clarified several important issues. These findings are summarized below in seven key conclusions and three practical recommendations.

CONCLUSIONS

1. A collection consisting of 76 pure cultures was created from samples taken from Azerbaijan's oil-contaminated, highly saline, thermal water sources, and subtropical forests with high humidity. Of these, 64 cultures belonged to micromycetes and 12 to macromycetes[1-5, 7-8].

2. It was determined that 75% of the micromycete strains are mesophilic, favoring moderate temperatures, while 25% are thermotolerant, capable of surviving at temperatures above 50°C. It was further established that the enzymatic activity of micromycetes ranges between 25.0% and 73.4%, and for macromycetes between 25.0% and 100%, including hydrolases (endo-glucanase, protease, pectinase, amylase, xylanase, and lipase) and oxidases (laccase and peroxidase)[3, 5-6].

3. *Aspergillus candidus*, *Chaetomium thermophile*, *Penicillium resticulosum*, *Humicola lanuginosa*, and *Mucor hiemalis* fungi were identified as promising producers of hydrolase-type enzymes. *Bjerkandera adusta* produces a balanced complex enzyme system, Trametes hirsuta is notable for oxidase production, and Trichoderma viride is a promising endo-glucanase producer [8-10].

4. Approximately 53.1% of micromycetes and 58.3% of macromycetes exhibited antibiotic activity against test cultures such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*. This activity was most pronounced in *Aspergillus flavus*, *Chaetomium globosum*, *Penicillium chrysogenium*, *P. citrinum*, *Trametes hirsuta*, and *Trichoderma viride* [5, 9].

5. All selected strains constitutively synthesize proteases;

other hydrolases in micromycetes are also constitutively synthesized. Endo-glucanase synthesis in *Trichoderma viride* and macromycetes occurs via an inducible mechanism and is regulated by catabolite repression. Oxidase synthesis in macromycetes follows classical (Jakob-Mone model) constitutive synthesis and, according to unitary theory, inducible synthesis [3, 6, 9, 11].

6. A weak but positive correlation exists between the degree of polymerization of carbon sources and enzyme secretion outside the cell in all selected strains. Nitrate forms of nitrogen are more effective in micromycetes, while ammonium forms are more effective in macromycetes [6, 9-10].

PRACTICAL RECOMMENDATIONS

1. The data obtained on the cultural-morphological and physiological-biochemical characteristics of microorganisms isolated from various extreme environments are important for the identification and differential use of these cultures. This information facilitates the prediction and isolation of new producers of biologically active metabolites (BAMs) active across a broad pH and temperature range.

2. The collection of natural strains isolated as active producers of biologically active substances from various extreme environments represents a valuable genetic resource for fundamental and applied biotechnological research.

The ecosystems affected by salt, petroleum pollution, and other extreme factors are prime environments for studying the distribution of microorganisms, particularly fungi. Despite existing research, there remains a gap in evaluating the biotechnological potential of these extremophilic fungi, which this study has aimed to address. By assessing the enzymatic and antibiotic activities of fungal strains isolated from these extreme environments, this work lays the groundwork for biotechnological applications that could be of interest for agricultural and industrial uses.

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The dissertation defense will take place on **29 October 2025 at 11-00** at hours at the meeting of the One-time Dissertation Council BFD 1.07/1 operating under the Microbiology Institute of the Azerbaijan National Academy of Sciences.

Address: AZ1004, Baku city, A. Abbaszade St., 115.

The dissertation is available for review at the library of the Microbiology Institute of the Azerbaijan National Academy of Sciences.

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