

Biotechnological Evaluation of Fungal Strains Isolated from Extreme Habitats Based on Their Enzymatic and Antimicrobial Properties

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Abstract. This scientific investigation focuses on obtaining, cataloging in a culture collection, and biotechnologically evaluating fungal isolates retrieved from diverse ecological niches characterized by extreme environmental stress in Azerbaijan. These habitats encompass petroleum-contaminated soils, high-salinity terrains, mineralized thermal springs, and humid subtropical forest ecosystems. A total of 76 fungal isolates (comprising 64 micromycetes and 12 macromycetes) were successfully derived into pure cultures using approved protocols and methods. The enzymatic potential of these isolates—specifically hydrolytic (endo-1,4- β -glucanase, protease, pectinase, amylase, xylanase, lipase) and oxidative (laccase, peroxidase) activities—was evaluated. The enzymatic activities were assessed using spectrophotometric methods, while antimicrobial effects were evaluated through agar block and disc diffusion assays. Furthermore, their antagonistic effects against clinically relevant human pathogens (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*) were thoroughly examined, leading to the identification of potent producing strains. Systematic optimization of environmental factors and growth media parameters yielded an 11–20% enhancement in target metabolite biosynthesis.

Keywords: extreme habitats, micromycetes, hydrolases, oxidases, antimicrobial potency, metabolic regulation, bioprocess optimization, microbial biotechnology

Introduction

In the contemporary era, the exponential expansion of the global population necessitates innovative microbiological strategies to address critical challenges in food security, sustainable bioenergy supplies, healthcare, and pharmaceutical raw material generation (Kutu et al., 2019). Current forecasts project that agricultural and food demand will experience a nearly twofold increase in the upcoming decades (Pobiega et al., 2024). Consequently, discovering biologically active natural resources with enhanced biosynthetic capabilities represents a key priority in modern bioprocess engineering (Rappaport & Oliverio, 2023).

Compared to conventional plant and animal bioresources, micromycetes offer substantial advantages, including rapid growth kinetics, short generation cycles, and the capacity to bioconvert low-cost industrial by-products into valuable biomass and bioactive compounds (Pobiega et al., 2024).

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Despite the vast species diversity of the kingdom Fungi, only a minor fraction has been harnessed for industrial applications. Extremophilic micromycetes, which have evolved specialized physiological machinery to thrive under severe environmental conditions (such as heavy metal toxicity, hypersalinity, thermal stress, and extreme pH levels), serve as an extraordinary reservoir for stable functional enzymes and bioactive molecules (Rappaport & Oliverio, 2023; Mirzezade & Mammadova, 2026).

The territory of Azerbaijan exhibits remarkable physiogeographic and geochemical diversity (Mammadova et al., 2026a). Unique ecological niches—including hydrocarbon-polluted soils across the Absheron Peninsula, mineralized thermal springs, hypersaline inland lakes, and the humid subtropical forest ecosystems of Hirkan National Park—provide ideal environments for the evolution of specialized microflora. Fungal strains retrieved from these extreme habitats possess high physiological resilience, making their extracellular enzymes and secondary metabolites highly valuable for bioprocess development (Mammadova et al., 2026b; Mammadova et al., 2026a).

The primary goal of this research is the isolation of micromycete and macromycete strains from extreme Azerbaijani habitats, comprehensive profiling of their enzymatic and antimicrobial characteristics, and the assessment of their biotechnological application prospects.

Materials and Methods

Environmental samples were gathered from distinctive stressed habitats in Azerbaijan, including petroleum-hydrocarbon-contaminated soils, hypersaline soil-water environments, thermal mineral springs, and humid subtropical forest ecosystems located within Hirkan National Park.

A total of 100 specimens were collected per designated sampling site, cataloged, and conveyed to the laboratory within 2–6 hours for subsequent processing. During the isolation phase, 10% (w/v) soil suspensions were formulated in sterile distilled water and allowed to stand at ambient temperature for 2 hours. Serial dilutions (undiluted, 10^{-1} , and 10^{-2}) were subsequently inoculated onto solid growth media, specifically malt extract agar (MEA) and Sabouraud dextrose agar. Following an incubation period of 2–3 days, colonies were differentiated according to their macro-morphological traits, transferred onto fresh nutrient media, and sequentially subcultured to yield axenic biomass. Culture purity was routinely confirmed via optical microscopy (OMAX 40X–2500X), and taxonomic identification was executed using established mycological keys and authoritative determinative manuals (White et al., 1990).

Isolation and Cultivation: Pure fungal cultures were isolated by serial dilution plating on Czapek-Dox, Wort Agar, and Meat-Peptide Agar nutrient media.

Protease Activity Assay: Extracellular proteolytic activity was determined spectrophotometrically using hemoglobin as a substrate, following the principles of Anson's method.

Carbohydrase Analysis: Endo-1,4- β -glucanase and amylase activities were evaluated spectrophotometrically by measuring released reducing sugars via the Somogyi-Nelson colorimetric technique (Vermelho & Couri, 2013).

Oxidoreductase Determination: Laccase and peroxidase activities were quantified spectrophotometrically based on the oxidation rates of ABTS and guaiacol substrates (Bourbonnais & Paice, 1990).

Antimicrobial Potency Evaluation: Antagonistic performance against target microbial pathogens was evaluated using agar block assays and disc diffusion methods (Danquah et al., 2022).

Biometric and Statistical Processing: All analytical trials were conducted in 4 to 6 replicates, and the quantitative data were processed using standard statistical techniques (White et al., 1990).

Results and Discussion

Investigations yielded a total of 76 pure fungal isolates, consisting of 64 micromycetes and 12 macromycetes. Physiological profiling revealed that 75% of the micromycetes belonged to the mesophilic group, whereas 25% exhibited thermotolerant characteristics, retaining growth capacity at elevated temperatures (Table 1).

Table 1
Temperature tolerance of micromycetes isolated from diverse biotopes

No.	Collection site	Thermotolerants (II)*	Mesophiles (I)*	Total Isolated Strains (pcs) 4
1	Petroleum-contaminated soils	5	11	16
2	High-humidity subtropical forests	4	9	13
3	Thermal springs	3	3	6
4	Lake Masazir (Saline environment)	4	9	13
Total	All habitats	16	32	48

*Note. Group I represents mesophilic strains, and Group II represents thermotolerant strains.

In order to select the most relevant micromycete and macromycete strains, their enzymatic activities were evaluated according to internationally recognized methods. We selected the strains with the highest activity based on enzymatic activity analyses using determinative agents (Ismayilova et al., 2025a). The end of the tests and determinative methods revealed that (Kirk et al., 2008).

Selected High-Yielding Producer Strains:

- Hydrolytic Enzymes: *Aspergillus candidus* (Mazsazir Lake), *Chaetomium thermophile* (thermal springs), *Penicillium resticulosum* (Mazsazir Lake), *Humicola lanuginose* (thermal springs), and *Mucor hiemalis* (oil-contaminated soil) were selected as prominent producers of hydrolytic enzymes.
- Complex Enzymatic System: *Bjerkandera adusta* demonstrated strong, balanced expression of both hydrolytic and oxidative enzyme complexes (subtropical forests).
- Oxidases: *Trametes hirsuta* displayed remarkable potential for laccase and peroxidase production (subtropical forests).
- Endoglucanase: *Trichoderma viride* demonstrated high cellulolytic activity (subtropical forests).

Screening of the isolated strains for extracellular enzyme production yielded the following results:

- Hydrolytic and oxidative activities were detected in 25.0% to 73.4% of the micromycetes.
- Enzymatic activities were identified in 25.0% to 100% of the macromycete isolates.

Table 2*The characteristics of fungi based on their fermentative activity*

No.	Enzymes	Number of isolated/active	Percentage in total (%)		
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

	MACROMUCOTES
	MICROMUCOTES

Antimicrobial Activity Analysis

Additionally, we tested the most promising producers in terms of antimicrobial activity using the disk diffusion method and methods that have been adopted globally in laboratory protocols based on antagonistic capabilities against pathogenic test organisms (Barabadi et al., 2022).

The results of the tests revealed that:

53.1% of micromycete isolates and 58.3% of macromycete isolates exhibited noticeable antimicrobial effects against test strains (Table 3).

The most pronounced antagonistic action was observed in cultures of *Aspergillus flavus* (Masazir Lake), *Chaetomium globosum* (oil-contaminated soil), *Penicillium chrysogenum* (Thermal springs), *Penicillium citrinum* (Thermal springs), *Trametes hirsuta*, and *Trichoderma viride* (subtropical forests).

Table 3*Antimicrobial activity characteristics of the isolated strains*

No.	Sampling location	Isolated /Active strains		Percentage of strains in total (%)	
		Micromycetes	Macromycetes	Micromycetes	Macromycetes
1	Petroleum-contaminated soils	21/14		66.7	
2	Lake Masazir (Saline environment)	17/9	0	52.9	0
3	Thermal springs	9/4		44.4	
4	High-humidity subtropical forests	17/7	12/6	41.2	58.3
	Total	64/34	12/6	100/53.1	100/58.3

Optimization of Cultivation Parameters

To enhance the biosynthesis of targeted enzymes and secondary metabolites in selected producer strains, culture conditions were systematically adjusted. Although an organism's genetic framework fundamentally governs the biosynthesis of any cellular metabolite, environmental parameters are equally critical in determining metabolic yields. Accordingly, this phase of the study focused on

defining the optimal growth medium to maximize the fermentative activity of the selected high-producing strains (Abdel-Azeem et al., 2020).

We systematically optimized key nutritional and physical parameters—specifically carbon and nitrogen substrates, cultivation modes, incubation temperature, pH levels, inoculum preparation protocols, and propagation period (Ismayilova et al., 2025b). The findings revealed that while certain regulatory conditions for fermentative expression were uniform across all evaluated fungal strains, other parameters exhibited distinct strain-specific variations (Abdel-Azeem et al., 2020).

Influence of Carbon Source: A positive correlation was identified between the degree of polymerization of the carbon substrate and extracellular enzyme secretion (Mammadova et al., 2026).

Role of Nitrogen Origin: Nitrate nitrogen sources were more effective for micromycete growth, whereas ammonium nitrogen compounds favored macromycetes.

Biosynthetic Yields: Optimization strategies resulted in a 15–20% increase in target product yield for Group 1 strains and an 11–17% increase for Group 2 strains.

Mechanistic evaluations confirmed that protease expression is operates via a constitutive pathway, whereas cellulase biosynthesis is inducible and regulated through catabolite repression.

Conclusion

The empirical findings expand current scientific understanding regarding the physiological and biosynthetic capabilities of fungal strains isolated from extreme environments. Promising strains—such as *Trichoderma viride*, *Trametes hirsuta*, *Aspergillus candidus*, and *Penicillium* species—represent valuable bioresources for the industrial-scale formulation of commercial enzyme preparations and bioactive compounds.

1. A total of 76 pure fungal cultures (64 micromycetes and 12 macromycetes) were isolated from stressed ecological habitats in Azerbaijan, with 25% of the micromycetes classified as thermotolerant, while mesophiles accounted for 75% and true thermophiles were not detected.
2. Hydrolytic and oxidative enzyme activities were observed in 25.0–73.4% of micromycetes and 25.0–100% of macromycetes.
3. Over 50% of the isolates demonstrated antimicrobial inhibition against pathogenic *E. coli*, *P. aeruginosa*, *S. aureus*, and *C. albicans* strains.
4. Modulation of growth parameters enhanced target metabolite accumulation by 11–20%.
5. Regulatory pathways (constitutive vs. inducible) and substrate dependencies governing fungal enzyme expression were established.
6. Optimization of cultivation parameters to enhance fermentative expression yielded a 15–20% increase in output, whereas refining the conditions for antimicrobial activity produced an 11–17% enhancement.

Conflict of Interest

The authors declare no competing interests that could be perceived as influencing the research or its publication. L.B. is an Assistant Editor of the journal. She was not involved in the editorial evaluation or publication decision concerning this manuscript.

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