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EMC'26

5th INTERNATIONAL EURASIAN MYCOLOGY *Congress*

Book of Proceedings and Abstracts



5th INTERNATIONAL EURASIAN MYCOLOGY CONGRESS (EMC'26)

5. ULUSLARARASI AVRASYA MİKOLOJİ KONGRESİ (EMC'26)

BOOK OF PROCEEDINGS AND ABSTRACTS

TAM METİN VE ÖZET KİTABI

September 01-03, 2026

Trakya University

Edirne-Türkiye

5th INTERNATIONAL EURASIAN MYCOLOGY CONGRESS (EMC'26)

5. ULUSLARARASI AVRASYA MİKOLOJİ KONGRESİ (EMC'26)

EDITOR OF BOOK OF PROCEEDINGS AND ABSTRACTS

Assoc. Prof. Dr. Halide KARABIYIK

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5. ULUSLARARASI AVRASYA MİKOLOJİ KONGRESİ (EMC'26)

September 01-03, 2026
Tuesday, Wednesday, Thursday
Trakya University

Book of Proceedings and Abstracts

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5th International Eurasian Mycology Congress 2026 (EMC'26)
FACE TO FACE PROGRAMME

01.09.2026 - Tuesday		
08.30-10:00	**Registration**	***Balkan Congress Center***
10:00-11:00	**Opening Ceremony**	
11:00-11:30	***C O F F E E B R E A K***	
11:30-12:30	**OPENING LECTURES (Balkan Congress Center-Conference Hall)**	
	Prof. Dr. Ahmet ASAN (Honorary President)	
	Prof. Dr. Ertuğrul SESLİ (Guest of Honor)	
	Plenary Session	**Chair:Prof. Dr. Ahmet ASAN** **Prof. Dr. Ertuğrul SESLİ**
	Invited Speaker (Balkan Congress Center-Conference Hall)	
12:30-13:00	***Prof. Dr. Ertuğrul KAYA*****,** Niğde Ömer Halisdemir Üniversitesi – TR ***Title*****:** Deadly Mushroom Poisonings: A Silent and Preventable Public Health Threat	
13:00 – 14:00	***L U N C H***	
	Session 1 **Medical and Veterinary Mycology** **Chair: Prof. Dr. Hossein ZARRINFAR** **Assoc. Prof. Dr. İskender KARALTI** **(Hall B2)**	**Session 2** **Fungal Systematics, Taxonomy and Nomenclature** **Chair: Prof. Dr. Gıyasettin KAŞIK** **Prof. Dr. Yusuf UZUN** **(Hall B3)**
14:00-14:15	Özleyiş Konyalı, Gülcan Kuyucuklu-Kazan, Fatma Kaynak Onurdağ Thematic and methodological analysis of postgraduate theses on *Candidozyma auris* in Türkiye	Şanlı Kabaktepe, Ali Kanat, Ilgaz Akata, Şükrü Karakuş, Ergin Şahin, Beste Gizem Özbey Two new records of powdery mildew fungus for Türkiye.
14:15-14:30	*NOT PRESENTED*	Yusuf Uzun, Sedat Kesici, Cemil Sadullahoğlu The new record from Hakkâri (Türkiye) *Pachyella hydrophila* (Sacc.) Pfister
14:30-14:45	Hossein Zarrinfar, Saba Takallou, Elham Elham Einafshar, Sahar Nikrooz, Amirreza Talebi, Mona Kabiri, Shaghayegh Moghadam Investigation and comparison the antifungal properties of graphene oxide, silver alumina and silver-alumina graphene oxide against clinical isolates of *Aspergillus*	Liz Tatiana Velasco Ospina, Ilgaz Akata, Eda Kumru Systematics and ecology of the genus *Geastrum*: A comprehensive review
14:45-15:00	Betül Günaydın, Hüseyin Haydar Kutlu, Yekta Özkılıç, Mine Aydın A report of a rare yeast from a tertiary care hospital: *Petasospora fabianii*	Mustafa Emre Akçay, İsmail Acar *Pholiotina mediterranea* (Bolbitiaceae, Agaricales): First record for the mycobiota of Türkiye confirmed by morphological and ITS sequence analyses
15:00-15:15	Aras Fahrettin Korkmaz Methods used for the identification of *Candida* spp. and changing taxonomy	Esra Gül, Young-Joon Choi, Fabian Runge, Marco Thines On the host range and diversity of the *Hyaloperonospora brassicae* complex
15:15-15:30	Esra Özkaya, Metin Sancaktar, Aysun Küçükali, Celal Kurtuluş Buruk, Ilknur Tosun Clinical distribution and antifungal susceptibility profiles of *Pichia kudriavzevii* (formerly *Candida krusei*) isolates: A nine-year retrospective evaluation	*NOT PRESENTED*
15.30 – 16.00	***C O F F E E B R E A K***	
	Plenary Session	**Chair: Prof. Dr. Rasime DEMİREL**
	Invited Speaker (Balkan Congress Center-Conference Hall)	
16:00-16:30	***Prof. Dr. Donald H. PFISTER,*** Harvard University - USA ***Title:*** Fungaria in a changing world (online)	
	Session 3 **Artificial Intelligence, Image Analyses and Digital Mycology** **Chair: Prof. Dr. Şanlı KABAKTEPE** **Assist. Prof. Dr. Şafak YILDIRIM** **(Balkan Congress Center-Conference Hall)**	
16:30-16:45	*NOT PRESENTED*	
16:45-17:00	Faruk Ayata, Ebubekir Seyyarer, İsmail Acar Classification of macrofungal genera from basidiospore micrographs using CLAHE-enhanced DenseNet121	
17:00-17:15	Ebubekir Seyyarer, Faruk Ayata, İsmail Acar Hybrid CNN-transformer architecture for automatic classification of mushroom species using deep learning	
17:15-17:30	İjlal Ocak Slime moulds as a universal model for minimal cognition, decentralized computing, and bio-industrial sustainability	
19.00	***O P E N I N G C O C K T A I L***	

02.09.2026 - Wednesday		
	Session 4 **Applied and Industrial Mycology** **Chair: Prof. Dr. Hacı Halil BIYIK** **Prof. Dr. Esra ERSOY ÖMEROĞLU** **(Hall B2)**	**Session 5** **Fungal Systematics, Taxonomy and Nomenclature / Fungal Phylogenomics and Molecular Evoluation / Macrofungal Diversity, Ecology and Biogeography** **Chair: Prof. Dr. Hatıra TAŞKIN** **Dr. Juan Carlos ZAMORA** **(Hall B3)**
09:30-09:45	*NOT PRESENTED*	*NOT PRESENTED*
09:45-10:00	*NOT PRESENTED*	*İjlal Ocak* Ecological niche breadth and substrate specialization of Myxomycetes on Anatolian black pine (*Pinus nigra* var. *pallasiana*): A multi-regional comparative analysis
10:00-10:15	*NOT PRESENTED*	*NOT PRESENTED*
10:15-10:30	*Büşra Altınsoy*, Süleyman Bayramoğlu Mycelium-based biocomposites: An evaluation for passive house applications	*Beste Gizem Özbey*, Ilgaz Akata, Mustafa Sevindik Effects of mycorrhizal symbiosis on soil water retention and water footprint in agricultural water management
	10.30 – 11.00 C O F F E E B R E A K	
	Plenary Session	**Chair: Assoc. Prof. Dr. Teodor DENCHEV**
	Invited Speaker (Balkan Congress Center-Conference Hall)	
11:00-11:30	***Prof. Dr. Hatıra TAŞKIN***, Çukurova University – TR ***Title:*** The Current State of *Morchella* Systematics: Unravelling Complexity in the True Morels	
	Session 6 **Medical and Veterinary Mycology** **Chair: Prof. Dr. Hüseyin GÜDÜCÜOĞLU** **Prof. Dr. Özlem ABACI GÜNYAR** **(Hall B2)**	**Session 7** **Plant Pathology and Agricultural Mycology** **Chair: Prof. Dr. Semra HASANÇEBİ** **(Hall B3)**
11:30-11:45		
11:45-12:00	Esra Özkaya, Metin Sancaktar, *Aysun Küçükali*, Celal Kurtuluş Buruk, İlknur Tosun Etiological distribution of fungal keratitis isolates: A 10-year experience of a tertiary care university hospital in a temperate climate	*Laila Bounar* Isolation and microbiological characterization of fungal pathogens infecting Moroccan avocado trees
12:00-12:15	*Ezgi Şababoğlu Baytaroğlu*, Merve Aydın, Emine Nedime Korucu, Ali Öztürk, Mustafa Emin Oz, Yusuf Sinan Şirin *Cryptococcus* species isolated from the external auditory canal in dogs and cats: Commensal flora or opportunistic pathogens?	Saba Amani Ghadim, *Rasime Demirel* Seasonal microfungal biodiversity assessment of fallow soils in Turkey's hop-growing region
12:15-12:30	*Handan Kurtulmuş Sancak*, Gülşah Çobanoğlu, Elif Çalışkan Salihi, Erkan Rayaman, Turgut Taşkın Development and characterization of *Usnea florida* extract-loaded sodium alginate hydrogel film with antimicrobial and antioxidant activities	*Bilge Irmak Özdinç*, Hüseyin Arasan, Ömer Faruk Altıbaş, Serhan Durgun, Semra Hasançebi PCR-based detection of *Macrophomina phaseolina* causing charcoal rot of sunflower
12:30-12:45	*Taner Tarladaçalışır*, Gülcan Kuyucuklu-Kazan Species distribution and antifungal susceptibility profiles in clinical *Candida* Isolates: A single-center experience from Edirne	*Fatma Akdeniz* New locality record of *Puccinia marrubii* (Pucciniaceae) on *Marrubium globosum* subsp. *globosum* (Lamiaceae) from Antalya, Türkiye
	13:00 – 14:00 L U N C H	
	Plenary Session	**Chair: Prof. Dr. Ilgaz AKATA**
	Invited Speaker (Balkan Congress Center-Conference Hall)	
14:00-14:30	***Assoc. Prof. Dr. Teodor DENCHEV,*** Bulgarian Academy of Sciences - BG ***Title:*** Overview of the Anthracoideaceae (Ustilaginales) – exploring the generic boundaries and host specificity	
	Session 8 **Medical and Veterinary Mycology** **Chair: Prof. Dr. Neşe KAKLIKKAYA** **Assist. Prof. Dr. Tülay BİCAN SÜERDEM** **(Hall B2)**	**Session 9** **Fungal Symbioses: Mycorrhizae, Lichens and Associated Interactions / Plant Pathology and Agricultural Mycology** **Chair: Prof. Dr. Gülşah ÇOBANOĞLU** **Assoc. Prof. Dr. Duygu KADAİFÇİLER** **(Hall B3)**
14:30-14:45	*Alparslan Semih Salan*, Suzan Ökten In vitro antifungal activity of *Aronia melanocarpa* extract alone and in combination with fluconazole against *Candida albicans*	*Gıyasettin Kaşık*, Sinan Alkan, Ibrahim Furkan Koç, Hediye Nur Demir Investigation of the utilization possibilities of corn cobs in *Pleurotus ostreatus* cultivation
14:45-15:00	*Zeynep Yazgan*, Gökhan Aygün, Nuri Kiraz, Zekayi Kutlubay Species distribution, species characterization, and antifungal susceptibility of clinical dermatophyte isolates	*Ayşe Betül Konar Korkmaz*, Gülşah Çobanoğlu Biomonitoring of atmospheric microplastics in İstanbul's green areas using *Xanthoria parietina*: A multi-analytical approach
15:00-15:15	*Gamze Altıntaş Kazar* Chaxines from the edible mushroom *Agrocybe chaxingu* engage the RORγt inverse-agonist pocket: an in silico docking and ADMET study	*Ezgi Özen*, Gülşah Çobanoğlu, Bora Garipcan, Pervin Rayaman Antifungal activity of acetone extracts from lichens incorporated into PVA-based polymeric membranes
15:15-15:30	*Bengi Aldı*, Bahar Gökçen, Özlem Abacı Günyar, Burak Durmaz Rodlet- and DHN-melanin-associated gene expression profiles and cytotoxicity in clinical *Aspergillus fumigatus* strains	*Gülbeden Mutlu*, Halide Karabıyık Soil microfungi as biocontrol and plant growth-promoting agents: An agroecological approach
	15:30 – 16:00 C O F F E E B R E A K	
	Plenary Session	**Chair: Prof. Dr. Hatıra TAŞKIN**
	Invited Speaker (Balkan Congress Center-Conference Hall)	
16:00-16:30	***Prof. Dr. Juan Carlos ZAMORA,*** Jardin Botanique de Genève, Switzerland - CH ***Title:*** Recent advances in the systematics of the genus *Geastrum*: where are we?	

	Session 10 **Artificial Intelligence, Image Analyses and Digital Mycology** **General Mycology** **Chair: Assoc. Prof. Dr. İsmail ACAR** **Assist. Prof. Dr. Aras Fahrettin KORKMAZ** **(Balkan Congress Center-Conference Hall)**
16:30-16:45	*Onur Kırdök*, Sultan Kübra Toker, Muhammet Aydoğdu From organic waste to mycelium biomaterials: Designing industrial symbiosis
16:45-17:00	*Selma Vuruşaner* The past, present, and future of fungal computers
17:00-17:15	*Mustafa Yıldız* A bioinformatics-based approach for interactive PCR-RFLP assay design
17:15-17:30	*Süleyman Bayramoğlu* A new future flourishing in nature: Mycelium, sustainable living, and human-centered education
17:30-18:30	**MİKOLOJİK ARAŞTIRMALAR DERNEĞİ (MİKODER) TOPLANTISI**

20:00 G A L A D I N N E R

03.09.2026 – Thursday

	Session 11 Applied and Industrial Mycology **Chair: Prof. Dr. İjlal OCAK** **Prof. Dr. Burhan ŞEN** **Hall B2**	**Session 12** Fungal Natural Products and Chemical Ecology Medical and Veterinary Mycology **Chair: Prof. Dr. Nükhet Nilüfer ZORBA** **Assoc. Prof. Dr. Gülay GİRAY** **Hall B3**
09:00-09:15	*Hatice Yıldız Acar*, Hacer Sert Breaking the fungal shield: A systematic review on the role of boron derivatives in biofilm inhibition and dispersal	*Yüksel Gezgin*, Beyza Efe Çiğdem, Ayşegül Yoltaş, Pelin Sağlam Metiner, Zeycan Nurçe, Sultan Gülçe-İz, İsmail Hakkı Akgün Isolation, structural elucidation, and biological evaluation of secondary metabolites from *Aspergillus* sp. isolate 1352
09:15-09:30	*Deniz Yüksel Yence* Antibiofilm effects of endophytic fungal secondary metabolites against *Staphylococcus aureus*	*Gülcan Kuyucuklu-Kazan*, Kültüral Güngör, Burak Küçük First detection of *Candida auris* (*Candidozyma auris*) candidemia at Kırklareli Training and Research Hospital: A three-case experience
09:30-09:45	*Bahar Gökçen*, Bengi Aldı, Özlem Abacı Günyar, Burak Durmaz Molecular and functional characterization of ribotoxin-producing filamentous fungi recovered from diverse environmental sources	*Gülşah Gedik* Sustainable use of mushrooms in pharmaceutical technology
09:45-10:00	*Senem Öztürk Köse*, Hacı Halil Bıyık, Mehtap Kılıç Eren, Esin Poyrazoğlu, Hatice Pilevneli Evaluation of the biodegradation of aflatoxin B1 and aflatoxin G1 by optimization of *Zygosaccharomyces rouxii* HBB542 Supernatant	*Yüksel Gezgin*, Sibel Arslan Gültaş, Cemre Ünver Korkmaz, Pelin Sağlam Metiner, Zeycan Nurçe, Ayşegül Yoltaş, Sultan Gülçe-İz, İsmail Hakkı Akgün Bioactivity-guided isolation and characterization of *Aspergillus brasiliensis* metabolites: Assessment of biological activities
10:00-10:15	*Yalçın Güçer*, Burak İşçimen Effects of Mold Fermentations on Food Quality	*Melek Tikveşli*, Halide Karabıyık, İrem Ürgün Hatikler, Canan Eryıldız, Hüseyin Güdücüoğlu Evaluation of the distribution of environmental saprophytic fungi and yeasts isolated from clinical samples
	10:15 – 10:45 C O F F E E B R E A K	
	Plenary Session	**Chair: Prof. Dr. Suzan ÖKTEN**
	Invited Speaker (Balkan Congress Center-Conference Hall)	
10:45-11:15	***Prof. Dr. Macit İLKİT,*** Çukurova University - USA ***Title:*** Extreme Guests of Modern Homes – The Hidden World of Extremotolerant Fungi. – *NOT PRESENTED*	
11:15-12:30	**Closing and Award Ceremony / *Balkan Congress Center***	

13:00-17:00 LUNCH and SOCIAL PROGRAM (City Tour)

POSTER PRESENTATION PROGRAMME

No	**01 September 2026 (09:00-17:30) / Discussion: in 15:30-16:00 coffee break**
P1	*Teodor T. Denchev*, Cvetomir M. Denchev Phylogenetic analyses, morphological characteristics, and host specificity of a smut fungus on *Carex glacialis* (Cyperaceae) reveal a new species, Anthracoidea glacialis (Anthracoideaceae, Ustilaginales)
P2	*Radoslav Abrashev*, Ekaterina Krumova, Jeny Miteva-Staleva, Lyudmila Yovchevska, Vladislava Dishliyska, Boryana Spasova, Yana Gocheva, Maria Angelova, Hristo Najdenski Cellulose-degrading fungi from native environmental sources with potential to break down waste during manned spaceflights
P3	*Vladislava Dishliyska*, Lyudmila Yovchevska, Nedyalka Kasabova-Slavova, Neli Gogova, Jeny Miteva-Staleva, Radoslav Abrashev, Boryana Spasova, Maria Angelova, Ekaterina Krumova Antimicrobial potential of filamentous fungi isolated from Antarctic Soils on Livingston Island
P4	Lyudmila Yovchevska, Maria Gerginova, Vladislava Dishliyska, Galina Stoyancheva, *Ekaterina Krumova* Catechol biodegradation and antioxidant defense responses in halotolerant filamentous fungi

P5	*Elif Doğan*, Nükhet Nilüfer Zorba Molecular identification and thermal resistance of heat-resistant molds isolated from foods: Influence of pH on ascospore heat resistance
P6	José-Antonio Bonet, Josu G. Alday, Marta Rovira, Daniel Oliach, İsmail Şen, Armin Mešić, Jasnica Medac, Anton Brenko, Ivana Zegnal, *Hatıra Taşkın*, Gökhan Baktemur, Ecem Kara, Emir Demirsoy, Claudia Perini, Sylvie Guillerme, Elena Salerni, Yasmin Piñuela, Iván Franco, Pia Höfferle, Nejc Suban, Anze Japelj Coordinated optimization for sustainable Mediterranean agroforestry with truffles and high-value species (COSMOS-PRIMA project)
P7	*Goulsoum Ouzeir*, Gamze Altıntaş Kazar, Burhan Şen Mycelial pellet morphology and pigmentation of selected *Penicillium* and *Talaromyces* species vary with culture medium mineral composition
P8	*Büşra Altınsoy*, Esin Kasapoğlu An evaluation of biocomposite ınsulatıon panels produced from mushroom mycel on existing patents and industrial applicability potential
P9	*Neli Gogova*, Neli Vilhelmova-Ilieva, Venelin Hubenov, Boryana Spasova, Lyudmila Yovchevska, Vladislava Dishliyska, Alexandra Rangelova, Jeny Miteva-Staleva Antitumor, anriviral and anti-inflammatory potential of Antarctic fungal strains
P10	Elif Şengül, *Duygu Kadaifçiler* Comparison of the cellulase production capacity of *Cladosporium* sp. isolated from a historical manuscript under different culture media, pH, and temperature conditions
	02 September 2026 (09:00-17:30) / Discussion: in 15:30-16:00 coffee break
P11	*Hossein Zarrinfar*, Monireh Kazemi, Majid Darroudi, Zahra Sabouri, Sahar Nikrooz, Seyedehsan Alavi, Shaghayegh Moghadam Preparation of selenium nanoparticles in plant extract substrates and investigation of antifungal properties and anticancer effects of cell lines
P12	*Didem Tuncay* Comparative evaluation of laccase activities in different macrofungi collected from the Istranca forests and their application in amperometric biosensor design
P13	*Selma Maytalman*, Sinan Meriç, Alp Ayan, Çimen Atak, Süleyman Bayramoğlu, Büşra Altınsoy Optimizing substrate composition and mineral additives for *Ganoderma lucidum*-based biocomposite production
P14	*Idil Malcek* Evaluation of the relationship between epiphytic lichen diversity and air quality: A comparative study in Edirne and Çorlu urban areas
P15	Rüya Sessiz, Ahmet Alperen Canbolat, *Nükhet Nilüfer Zorba*, Sercan Karav In vitro evaluation of the antifungal activity of different Lactoferrin preparations against *Candida albicans*
P16	*Utku Güner* Aquatic fungal pathogens in experimental animal models
P17	Lyudmila Yovchevska, Galina Stoyancheva, Vladislava Dishliyska, Jeny Miteva-Staleva, Radoslav Abrashev, Maria Angelova, Yana Gocheva, Maria Gerginova, *Ekaterina Krumova* Cellular response of halotolerant fungi from the Bulgarian Black Sea region against salt stress
P18	Nurlan Amrahov, Afsana Mammadova, Ruhangiz Mammadova, Ziyaddin Mammadov, Aslı Bayer, *Esra Ersoy Omeroğlu* First occurrence of *Fusarium equiseti* infecting cotton (*Gossypium hirsutum* L.) in Azerbaijan
P19	*Sultan Kübra Toker,* Onur Kırdök Marine biomass valorization through fungal fermentation: A biostimulatory approach
P20	*Gizem Güler,* Rasime Demirel Evaluation of chitosan production potential of soil-borne Zygomycetes under different cultivation conditions
	03 September 2026 (09:00-17:30) / Discussion: in 10:15-10:45 coffee break
P21	*Dilara Çobanoğlu*, Halide Karabıyık Evaluation of the antifungal activity of clove, mustard, and black cumin seeds against phytopathogenic fungi
P22	*Şafak Yıldırım* Utilizing food ındustry waste through fungi: Green biotechnology applications
P23	*Burak İşçimen*, Yalçın Güçer Mold fermentation in professional kitchens and emerging gastronomic trends
P24	*Tuğba Korur Kolanlarlı*, Ahmet Asan Investigation of mycotoxin presence in some unpackaged spıces obtained from herbalists in Edirne province using the LC-MS/MS method
P25	*Muhammed Ali Boz,* Beren Ekin Demir, Tülay Bican Süerdem, Alper Tunga Akarsubaşı Benchmarking Evo 2 against experimental Fks1 fitness landscapes of echinocandin resistance
P26	*Tülay Bican Süerdem,* Ayhan Kastır Evaluation of antifungal potential of *Morinda citrifolia* L. (Noni) against dermatophytes
P27	*İrem Mart,* Tülay Bican Süerdem Bioactive components of *Auricularia auricula-judae* (Bull.) Quél. and its potential in wound healing: A current review

ONLINE PROGRAMME

	02.09.2026 **Session 13** **Oral Presentation** **Chair: Assist. Prof. Dr. Aras Fahrettin KORKMAZ** **Dr. Melek TİKVEŞLİ** **Medical and Veterinary Mycology**
10:00-10:10	*Ravil Huseynov* Diagnostic challenges in contemporary medical mycology: An integrated approach to invasive fungal infections
10:10-10:20	*Sevinc Aliyeva* , Sariyya Rahimova Epidemiology of dermatophyte infections in Azerbaijan: Species distribution, gender analysis and clinical correlation a retrospective microbiological assessment

10:20-10:30	*Dilruba Garashova*, Dilara Abdullayeva, Vugar Huseynov, Amalya Zeynalova From sputum culture to diagnosis: Multidisciplinary recognition of allergic bronchopulmonary aspergillosis
10:30-10:40	*Ismayıl Valiyev* Dermatophytes in medical mycology: Current approaches to clinical and laboratory diagnosis
10:40-10:50	*NOT PRESENTED*
10:50-11:00	*Eleonora Dubljanin*, Jovana Stojanovic, Isidora Vujcic Species distribution and antifungal susceptibility profile of *Aspergillus* bloodstream isolates from a critical care unit using EUCAST methodology
10:50-11.00	**DISCUSSION**
	Session 14 **Oral Presentation** **Chair: Assist. Prof. Dr. Aras Fahrettin KORKMAZ** **Dr. Melek TİKVEŞLİ** **General Mycology**
11:30-11:40	*NOT PRESENTED*
11:40-11:50	*Antony Ortiz-Suarez*, Slavomír Adamčík, Adriana Corrales Osorio Novel *Russula* species in Colombia reveal ecological and evolutive patterns related to host-symbiont interaction with Fagaceae family trees
11:50-12:00	*Mariana Carrillo*, Adriana Corrales Osorio, William Bravo, Lina Caro Use of native soil inoculum for the restoration of root-associated fungal communities in endangered *Juglans neotropica*
12:00-12:10	*Michelle Vera Castellanos*, Dabao Sun Lü, Håvard Kauserud, Inger Skrede Genomic consequences of postglacial secondary contact between diverged lineages of *Trichaptum abietinum* in Europe
12:10-12:20	*Eleonora Dubljanin*, Jovana Stojanovic, Isidora Vujcic Comparative diagnostic performance of *Aspergillus* Real-Time PCR, Galactomannan Antigen detection, and blood culture in intensive care unit patients with probable ınvasive pulmonary aspergillosis
12:20-12:30	*Şuheda Aldemir Terman*, İsmail Acar, Halide Karabıyık Two new records of *Helvella semiobruta* Donadini & Berthet and *Helvella varia* X.C. Wang & W.Y. Zhuang from Çanakkale, Türkiye
12:30-12.40	**DISCUSSION**

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INVITED SPEAKERS

The Current State of *Morchella* Systematic: Unravelling Complexity in the True Morels

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Abstract

Morchella is one of genus prized and consumed commonly for their distinctive aroma and taste. Despite significant progress in its cultivation, wild-harvested morel species remain to attract attention due to their rich flavor profiles, the fact that cultivation techniques cannot yet be applied to every species, and the ongoing inability to cultivate them in indoor environments. Significant progress has been made in morel systematics with the application of molecular analyses based on DNA sequence analysis. The use of just molecular and microscopic characters is insufficient for distinguishing between species, given the variability of morphological traits and the similarity of microscopic characteristics. Consequently, an integrative approach that simultaneously evaluates morphological, microscopic, ecological, geographical, and molecular data has been adopted. This approach has begun to reshape the systematics of the genus. Assessments—initiated on a large scale in North America, Türkiye, and China, and subsequently expanded across many other countries and regions—have revealed that the genus, previously thought to comprise only a few species, is actually highly diverse. These developments have led to the identification of synonymous species and the description of new ones, while also enriching our knowledge regarding host preferences and geographical distributions. Furthermore, researchers have identified morel species capable of emerging after fires and also the species suitable for cultivation, while also conducting in-depth studies into the genus's reproductive strategies. During this period, rare morel species were also identified, with recommendations offered to include some of them on the Red List. Suggestions regarding the origin of the *Morchella* have been offered, and efforts have been carried out to identify hotspots characterized by high species diversity. Detailed criteria were considered for species identification; innovative approaches were incorporated, such as addition of new microscopic criteria, photographing the specimens at various stages of maturity, documenting and photographing the ecological habitat detailed, and capturing SEM images of the spores.

Keywords: *Morchella*, morel, systematics, taxonomy

Recent advances in the systematics of the genus *Geastrum*: where are we?

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Abstract

The genus *Geastrum* Pers., known as earthstars, comprises a group of saprotrophic gasteroid fungi with a worldwide distribution. Known since the XVII century, it counts nowadays with over 120 accepted species, some of them poorly known and endangered. Many species are morphologically difficult to distinguish, and molecular tools are helping at discovering and describing many new taxa in the last decades, particularly in some tropical areas. Solving the identity of some broadly known names such as *G. triplex*, *G. saccatum*, and *G. javanicum* proved to be elusive unless abundant newly collected material for their respective type localities is available. On the other hand, dozens of validly published names have fallen into oblivion and newly described taxa sometimes lack the necessary comparisons with the information found in old literature and with fungarium specimens. Collaborative approaches involving integrative taxonomy and NGS of type specimens are shown as promising ways to build up new knowledge and speed up taxonomy without sacrificing accuracy, allowing thorough species descriptions and more stable systematics and nomenclature for future taxonomists.

Keywords: Geastraceae, integrative taxonomy, fungaria, nomenclature, protologues, types

Fungaria in a changing world

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Abstract

Mycological collections form the foundation for much of the basic research involving fungi. It is critical that collections are made that include adequate well documented material. The attention to elements associated metadata is essential -- this includes accurate locality and ecological data. Older collections often lack complete data on their specimen labels but such information can often be gleaned from study of the literature, diaries and other published and unpublished works. Well documented collections serve as voucher specimens for systematic, ecological and other studies and their deposition in permanent collections is essential to ensure the reliability and verifiability of research results. Conservation efforts depend on specimen data to determine abundance, distribution and other factors. The introduction of fungus specific terminology helps to highlight the importance of fungi in conservation discussions and assessments. The concept of the 3 Fs – Flora, Fauna and Funga – gives prominence to fungi as important elements in the ecosystem. Fungaria represent the fungus world as we know it and are the nucleus of work that goes beyond simply identification.

Keywords: Conservation, documentation, fungaria, taxonomy, voucher specimens

Overview of the Anthracoideaceae (Ustilaginales) – exploring the generic boundaries and host specificity

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Abstract

Anthracoideaceae was introduced in 1997 to accommodate the Anthracoidea species that have a unique type of two-celled, aerial basidium, with spores liberating directly into the air. Since then, many other genera have been transferred to this family, and in its current circumscription, Anthracoideaceae includes 19 genera. Species are parasites on Cyperaceae and Juncaceae, excluding five anamorphic species in Farysia. Our study of the Anthracoideaceae is based on analysis of the combined dataset of ITS, LSU, SSU, and TEF-1α sequence data, comparative morphology, and host specificity of the species. As a result of this study, we propose the description of a new genus of smut fungi, based on specimens from South America. Further, we discuss the boundaries of the currently recognized anthracoideaceous genera and their placement within the Ustilaginales.

This study was supported by the Bulgarian National Science Fund (Grant no. KP-06-N91/6/03.12.2025).

Keywords: Anthracoidea, phylogeny, smut fungi

Deadly Mushroom Poisonings: A Silent and Preventable Public Health Threat

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Abstract

Although mushroom poisoning is not very common, it can still be fatal today despite all treatments. Deaths due to mushroom poisoning still occur all over the world. While there is no cure, it is a preventable health problem. In fact, there are not many species of macrofungi that cause fatal poisoning: out of the approximately 140,000 fungal species identified worldwide, only about 40 are known to cause fatal poisoning. The species that causes the most deaths is *Amanita phalloides*. Approximately 100.000 cases of mushroom poisoning occur annually throughout the world (1.000-5.000 cases in Türkiye). Of these, approximately 500 people die (<%1). Of these, approximately 490 people die due to amatoxin-containing mushrooms (<%98). Of these, approximately 440 people die due to *Amanita phalloides* (<%90). Macrofungi contain various toxic chemical compounds that cause poisoning, such as amanitin, viroidin, orellanin, muscarine, and psilocybin. Mushroom poisonings are classified in two different ways based on the latent period or the type of toxic compound. Certain mushroom species containing amanitin, a cyclopeptide, the fungal toxin that causes the most deaths, include: *Amanita phalloides, Amanita verna, Amanita virosa, Amanita vidua, Amanita bisporigera, Amanita ocreata, Galerina marginata, Galerina sulciceps, Galerina venenata, Conocybe filaris, Lepiota brunneoincarnata, Lepiota brunneolilacea, Lepiota clypeolaria, Lepiota helveola, Lepiota subincarnata.* Amanitins, which have a cyclopeptide structure, are the mushroom toxins that cause the most deaths. When humans (and many animal species) consume mushrooms containing amanitins, the amanitin group of toxins (especially alpha-amanitin) is absorbed from the intestines and reaches the liver. The liver cannot detoxify these toxins, and alpha-amanitin halts protein synthesis in liver cells. If a sufficient amount of alpha-amanitin is ingested, the individual dies from liver failure. If a successful liver transplant is not performed, the person may lose their life within 4 to 9 days. There are various methods for the analysis of amanitin in mushroom samples and biological specimens. Approximately 50 grams of fresh *Amanita phalloides* mushroom (or 21 mg amatoxin) is lethal for an adult human. The lethal dose limit for humans is approximately 0.32 mg/kg of amatoxin. Specimens of the *Amanita phalloides* mushroom can resemble many other mushroom species, a situation that invites poisoning. Misconceptions and incorrect information about poisonous mushrooms can also increase the incidence of poisoning. Some rare incidents involving the misuse of such lethal mushrooms for the purpose of assassination have been reported. Sufficient effort must be made to prevent fatal mushroom poisonings, for which no adequately effective treatment has yet been found. Please note: You can eat all species of mushrooms; but some may be your last meal.

Keywords: Lethal mushroom intoxications, amanitin, *Amanita phalloides*

FULL TEXT

Etiological Distribution of Fungal Keratitis Isolates: A 10-Year Experience of a Tertiary Care University Hospital in a Temperate Climate

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Abstract

Fungal keratitis is a suppurative, sight-threatening corneal infection that requires timely mycological diagnosis and targeted therapy to prevent permanent visual loss. This nine-year retrospective study evaluated the overall etiological spectrum, and demographic characteristics of fungal pathogens isolated from corneal scrapings at a tertiary university hospital in a temperate, non-tropical region.

Corneal scraping specimens collected between 2017 and 2026 were retrospectively analyzed (single unique isolate per patient; duplicates excluded). Initial screening comprised direct microscopy and culture, followed by species-level identification using MALDI-TOF MS (Bruker Daltonics, Germany). Demographic data (sex) were recorded; descriptive statistics were applied.

Over nine years, 10 non-duplicate isolates from 10 patients (60% male, 40% female) were identified, consistent with the low incidence of keratomycosis in temperate climates. Filamentous fungi predominated (70%, n=7), followed by yeasts (30%, n=3). Among molds, *Fusarium* spp. were most frequent, including *Fusarium solani* (n=2) and *Fusarium proliferatum* (n=2), followed by *Aspergillus flavus* (n=1). Notably, *Scedosporium prolificans* (syn. *Lomentospora prolificans*) (n=1) and the black yeast-like *Aureobasidium pullulans* (n=1) were precisely identified. Yeast isolates comprised *Candida albicans* (n=2) and *Candida parapsilosis* (n=1). Temporal clustering was not observed given the low case numbers.

In keeping with non-tropical epidemiology, molds—particularly *Fusarium* species—remain the principal agents of keratomycosis in our setting. The identification of critically rare and highly drug-resistant opportunists such as *S. prolificans* and *A. pullulans* underscores that the clinical and scientific value of this series relies on its qualitative diversity. These findings demonstrate that continuous, long-term surveillance of local fungal etiologies is vital for expanding regional epidemiological data, informing accurate species identification, and guiding targeted empirical antifungal management.

Keywords: Fungal keratitis, corneal scraping, keratomycosis

Fungal Keratit İzolatlarının Etiyolojik Dağılımı: Ilıman İklimdeki Üçüncü Basamak Bir Üniversite Hastanesinin 10 Yıllık Deneyimi

Özet

Fungal keratit, kalıcı görme kaybını önlemek için zamanında mikolojik tanı ve hedefe yönelik tedavi gerektiren, süpüratif ve görmeyi tehdit eden bir kornea enfeksiyonudur. Bu on yıllık retrospektif çalışmada, ılıman ve tropikal olmayan bir bölgedeki üçüncü basamak bir üniversite hastanesinde kornea kazıntılarından izole edilen fungal patojenlerin genel etiyolojik dağılımı ve demografik özellikleri değerlendirilmiştir.

2017–2026 yılları arasında alınan kornea kazıntısı örnekleri geriye dönük olarak incelenmiştir (her hastadan tek izolat alınmış, tekrarlayan izolatlar dışlanmıştır). İlk değerlendirmede direkt mikroskopi ve kültür yapılmış, ardından izolatlar MALDI-TOF MS (Bruker Daltonics, Almanya) ile tür düzeyinde tanımlanmıştır. Demografik veri olarak cinsiyet kaydedilmiş ve tanımlayıcı istatistikler uygulanmıştır.

On yıllık dönemde 10 hastadan (%60 erkek, %40 kadın) tekrarlamayan 10 izolat tanımlanmıştır; bu sayı, ılıman iklimlerde keratomikozun düşük insidansıyla uyumludur. Filamentöz mantarlar (%70, n=7) mayalardan (%30, n=3) daha sık saptanmıştır. Küfler arasında en sık *Fusarium* türleri bulunmuş; *Fusarium solani* (n=2) ve *Fusarium proliferatum* (n=2) izolatlarını *Aspergillus flavus* (n=1) izlemiştir. Ayrıca *Scedosporium prolificans* (sin. *Lomentospora prolificans*) (n=1) ve siyah maya benzeri *Aureobasidium pullulans* (n=1) tür düzeyinde tanımlanmıştır. Maya izolatları *Candida albicans* (n=2) ve *Candida parapsilosis* (n=1) idi. Olgu sayısı düşük olduğundan zamansal kümelenme gözlenmemiştir.

Tropikal olmayan bölgelerin epidemiyolojisiyle uyumlu olarak, merkezimizde keratomikozun başlıca etkenleri küfler, özellikle *Fusarium* türleridir. *S. prolificans* ve *A. pullulans* gibi son derece nadir ve ilaçlara yüksek düzeyde dirençli fırsatçı etkenlerin saptanması, bu serinin klinik ve bilimsel değerinin niteliksel çeşitliliğinden kaynaklandığını göstermektedir. Bulgularımız, yerel fungal etkenlerin uzun süreli ve sürekli sürveyansının bölgesel epidemiyolojik verileri genişletmek, doğru tür tanımlamasını desteklemek ve hedefe yönelik ampirik antifungal tedaviyi yönlendirmek için gerekli olduğunu göstermektedir.

Anahtar Kelimeler: Fungal keratit, kornea kazıntısı, keratomikoz

Introduction

Fungal keratitis (keratomycosis) is a severe corneal infection that often results in blindness and loss of the eye. A systematic review estimated a minimum of 1,051,787 cases worldwide each year; in three case series, 8–11% of patients required removal of the eye, corresponding to an estimated annual loss of 84,143–115,697 eyes (Brown et al., 2021). More than 95% of cases are caused by *Fusarium*, *Aspergillus* and *Candida* species, with filamentous fungi responsible for most infections in tropical and subtropical climates and yeasts being more frequent in temperate climates (Brown et al., 2021).

This climate-based dichotomy has become less clear-cut. A London referral hospital reported an altered pattern with increasing filamentous fungal keratitis associated with contact lens wear (Ong et al., 2016), and a twelve-year Swiss series found moulds 1.8 times more common than yeasts in a predominantly

young, contact lens-wearing population (Bajka et al., 2024). In contrast, *Candida* species remained predominant among older patients with ocular surface disease, previous corneal surgery or topical steroid use in the United Kingdom and in humid temperate Galicia, Spain (Ting et al., 2021; Lamas-Francis et al., 2024).

In Türkiye, national estimates of the burden of fungal keratitis are derived from modelling rather than prospective surveillance (Hilmioğlu-Polat et al., 2019; Brown et al., 2021), and data on local etiology come from a limited number of regional series, mainly from İzmir, Adana and İstanbul (Yilmaz et al., 2007; Harbiyeli et al., 2022; Yarımada et al., 2022; Yıldız et al., 2023). In a national systematic review of infections due to rare moulds, the number of studies reporting such cases increased over time and the eye was the most frequently involved site (Gülmez, 2022). To our knowledge, the etiological profile of fungal keratitis has not been reported from the humid temperate Eastern Black Sea region. The aim of this study was to describe the etiological spectrum, species-level diversity and demographic profile of culture-proven fungal keratitis isolates recovered from corneal scrapings over a ten-year period at a tertiary university hospital in Trabzon, and to compare this profile with published Turkish and European series.

Materials and Methods

Study Design and Setting

This retrospective, descriptive, laboratory-based study was conducted at the Department of Medical Microbiology, Karadeniz Technical University Faculty of Medicine, a tertiary referral university hospital in Trabzon, in the Eastern Black Sea region of Türkiye. Laboratory records of corneal scraping specimens submitted between 2017 and 2026 were reviewed. Cultures yielding growth were eligible; growth assessed as contamination with normal skin flora bacteria was excluded. For fungal isolates, one isolate per patient was included and duplicate isolates were excluded. Clinical risk factors, treatment and outcome data were not part of this first-stage laboratory review.

Mycological Culture and Identification

Corneal scrapings were processed according to routine laboratory procedures and examined by direct microscopy. Corneal scraping material was inoculated onto blood agar, chocolate agar, eosin methylene blue agar, and Sabouraud dextrose agar using a C-shaped streaking technique. Culture media were incubated at 28 °C and 37 °C under appropriate atmospheric conditions, with fungal cultures observed for up to six weeks (Leck, 2009). Fungal growth was assessed on culture, and definitive species-level identification was performed using MALDI-TOF MS (MALDI Biotyper, Bruker Daltonics, Bremen, Germany). Additional confirmation at the national reference laboratory was obtained where applicable.

Data Collection and Analysis

Age, sex, requesting ward and year of isolation were retrieved from laboratory records. Age was expressed in completed years, calculated from the date of birth and the date of the laboratory request.

Categorical variables are presented as counts and percentages, and age as median and range. Owing to the small number of cases, no inferential statistical tests were applied. The proportion of fungal isolates was calculated relative to all culture-positive corneal scraping isolates rather than to all submitted specimens; it therefore represents an etiological share, not an incidence estimate.

Results

During the study period, 54 cultures from corneal scrapings yielded growth. Three were assessed as contamination with normal skin flora and excluded, leaving 51 culture-positive isolates: 41 (80.4%) bacterial and 10 (19.6%) fungal. The 10 fungal isolates were recovered from 10 different patients, all of whom were hospitalised in the ophthalmology ward; the laboratory request was issued on the day of admission in seven patients and within two days of admission in the remaining three.

Six patients (60%) were male and four (40%) were female. The median age at sampling was 65 years (range 37–86), and eight patients (80%) were aged 60 years or older. Fungal isolates were detected in eight of the ten calendar years, with a maximum of two isolates per year (2018 and 2021) and none in 2022 or 2024 (Figure 1).

Filamentous fungi accounted for seven isolates (70%) and yeasts for three (30%) (Table 1, Figure 2). *Fusarium* was the most frequent genus (4/10, 40%): two isolates were identified as *Fusarium solani* (Mart.) Sacc. (FSSC) and two as *Fusarium proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg (FFSC). The remaining moulds were *Aspergillus flavus* Link (n=1), *Scedosporium prolificans* (Hennebert & B.G. Desai) E. Guého & de Hoog (syn. *Lomentospora prolificans* Hennebert & B.G. Desai; Kidd et al., 2023) (n=1) and the black yeast-like fungus *Aureobasidium pullulans* (de Bary & Löwenthal) G. Arnaud (n=1). The yeast isolates were *Candida albicans* (C.P. Robin) Berkhout (n=2) and *Candida parapsilosis* (Ashford) Langeron & Talice (n=1).

Five of the seven mould infections occurred in men, whereas two of the three yeast isolates were from women. The median age was 65 years in both the mould group (range 37–83) and the yeast group (range 63–86). The youngest patient (37 years) had *F. proliferatum* keratitis, and the two rare opportunists, *S. prolificans* (2017) and *A. pullulans* (2026), were recovered from men aged 69 and 83 years, respectively.

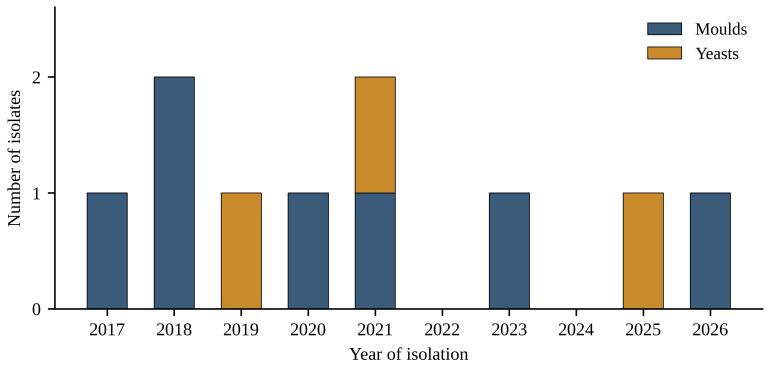


Figure 1. Annual distribution of fungal keratitis isolates by morphological group, 2017–2026.

Table 1. Culture-proven fungal keratitis isolates recovered from corneal scrapings, 2017–2026 (n=10)

Case	Isolate	Group	Age (years)	Sex	Year
1	*Scedosporium prolificans*	Mould	69	M	2017
2	*Fusarium solani* (FSSC)	Mould	62	M	2018
3	*Aspergillus flavus*	Mould	49	M	2018
4	*Candida albicans*	Yeast	86	F	2019
5	*Fusarium proliferatum* (FFSC)	Mould	65	M	2020
6	*Fusarium proliferatum* (FFSC)	Mould	37	F	2021
7	*Candida albicans*	Yeast	65	F	2021
8	*Fusarium solani* (FSSC)	Mould	68	F	2023
9	*Candida parapsilosis*	Yeast	63	M	2025
10	*Aureobasidium pullulans*	Mould (melanised)	83	M	2026

FSSC: *Fusarium solani* species complex; FFSC: *Fusarium fujikuroi* species complex; M: male; F: female. Age is given in completed years at the laboratory request date.

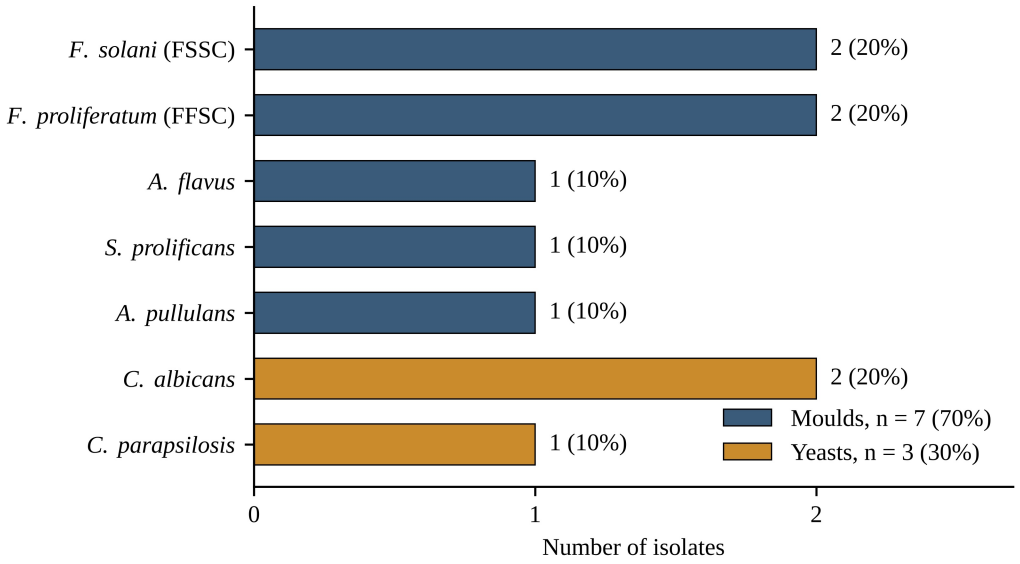


Figure 2. Species-level distribution of fungal keratitis isolates (n=10) by morphological group. FSSC: *Fusarium solani* species complex; FFSC: *Fusarium fujikuroi* species complex.

Discussion

In this ten-year laboratory series from a humid temperate region of Türkiye, moulds accounted for 70% of fungal keratitis isolates and *Fusarium* was the leading genus. The small absolute number of fungal isolates is consistent with the low incidence of keratomycosis in temperate climates (Brown et al., 2021), but fungi constituted 19.6% of culture-positive corneal scraping isolates. Proportions of fungal isolates of similar or greater magnitude have been reported from Türkiye: 16.7% of culture-positive patients at a tertiary centre in İstanbul in 2013–2023 (Yıldız et al., 2023), 22.3% of culture-positive keratitis in İzmir in 1990–2005 (Yilmaz et al., 2007), 35.2% of culture-positive patients at another İzmir referral

centre in 2012–2019 (Yarımada et al., 2022), and 36.7% of culture-positive infections among patients aged 60 years or older at the latter centre in 2012–2025 (Karahan et al., 2026). These values are well above the 3–9% of microbial keratitis cases attributed to fungi in European series, as summarised by Lamas-Francis et al. (2024). Because our denominator was the culture-positive isolate pool rather than all patients with clinically diagnosed microbial keratitis, these figures are not directly comparable and should not be interpreted as incidence.

At the genus level, our findings closely resemble earlier data from the Aegean region (Table 2). In the İzmir series of Yilmaz et al. (2007), *Fusarium* spp. (n=25), *Candida* spp. (n=15) and *Aspergillus* spp. (n=10) accounted for all 50 fungal isolates, as summarised by Hilmioğlu-Polat et al. (2019); these counts correspond to a mould-to-yeast ratio of 70:30, identical to that of our series. More recently, *Fusarium* spp. ranked among the three most common pathogens (11.2%) at an İzmir referral centre, and 75% of the patients with fungal keratitis had a history of ocular trauma with organic material (Yarımada et al., 2022). In the Çukurova region, described by Harbiyeli et al. (2022) as subtropical and agricultural, moulds caused 34 of 38 fungal keratitis cases (89.5%) and 22 of 26 culture-positive cases, but *Aspergillus* rather than *Fusarium* was the leading genus (11 of 26 culture-positive cases, followed by *Fusarium* in 8); the mean patient age in that series (44.9 years) was two decades lower than the median age in ours, and trauma was the leading predisposing factor in patients younger than 40 years (Harbiyeli et al., 2022). A different picture has been reported from a tertiary centre in İstanbul, where the 16 fungal isolates recovered from 15 inpatients in 2013–2023 comprised nine yeasts (*C. parapsilosis* and *C. albicans*, four each, and one further *Candida* isolate) and seven filamentous fungi, including two *F. solani* isolates, a mould-to-yeast ratio of 7:9 (Yıldız et al., 2023). Corneal transplantation was the most common risk factor in that cohort (42.2%), in which the mean age of culture-positive patients was 64 years, but the proportion of fungal infections did not differ significantly between patients with corneal transplantation and those with other risk factors (bacteria caused 86.8% and 80.8% of infections, respectively; p=0.445) (Yıldız et al., 2023). The authors related their fungal keratitis rate to the small number of referrals from rural areas (Yıldız et al., 2023), and a predominantly urban referral population with less exposure to organic material could also contribute to the lower share of mould infections; risk factors, however, were not reported separately for fungal cases. Methodological differences may play a part as well, because cultures in that study were reported as incubated for 48 h (Yıldız et al., 2023), whereas up to two weeks may be needed before the absence of fungal growth can be confirmed (Brown et al., 2021). Yarımada et al. (2022) related the high proportion of fungal keratitis in their series to the widespread agricultural activity in Türkiye. Because risk factors were not recorded in our study, a similar role for exposure to organic material in the Eastern Black Sea region, where tea and hazelnut cultivation are widespread, remains a hypothesis to be tested in studies that collect occupational and trauma histories.

Table 2. Comparison of the present series with selected published fungal keratitis series

Setting (study period); source	Study population	Age (years)	Moulds : yeasts	Leading fungal agent
Trabzon, Türkiye (2017–2026); present study	10 fungal isolates	Median 65	70% : 30%	*Fusarium* spp. (4/10)
İzmir, Türkiye (1990–2005); Yilmaz et al., 2007	50 fungal isolates (22.3% of culture-positive)	Mean 54.1[b]	70% : 30%[a]	*Fusarium* spp. (25/50)[a]
İzmir, Türkiye (2012–2019); Yarımada et al., 2022	50 fungal patients (35.2% of culture-positive)	Mean 51.4[b]	Not reported	*Fusarium* spp. (11.2% of culture-positive)
İstanbul, Türkiye (2013–2023); Yıldız et al., 2023	15 fungal patients, 16 isolates (16.7% of culture-positive)	Mean 64[b]	43.8% : 56.3% (7 : 9 isolates)	*Candida* spp. (9/16)
Adana, Türkiye (2012–2018); Harbiyeli et al., 2022	38 cases (26 culture-positive)	Mean 44.9	89.5% : 10.5% (culture-positive 22 : 4)	*Aspergillus* spp. (11/26)
Greece, 16-year nationwide survey; Mpakosi et al., 2022	35 filamentous cases	Median 48	Moulds only (by design)	*Fusarium* spp. (61.8%)
Galicia, Spain (2010–2020); Lamas-Francis et al., 2024	77 cases	Median 68	44.2% : 55.8%	*Candida* spp.
Zurich, Switzerland (2012–2023); Bajka et al., 2024	206 patients	Median 38	1.8 : 1	*Candida* spp., then *Fusarium* spp.
United Kingdom (2011–2020); Ting et al., 2021	117 patients (52 culture-positive)	Mean 59.0	Yeast-predominant	*Candida* spp. (62.3% of isolates)

[a]Genus counts as summarised by Hilmioğlu-Polat et al. (2019); the mould-to-yeast ratio was derived from these counts. [b]Age of the whole microbial keratitis or culture-positive cohort, not of fungal cases alone. Denominators (isolates, cases or patients) differ between studies.

By contrast, several temperate European series have been yeast-predominant, particularly in older patients. In a ten-year series from the United Kingdom, *Candida* species accounted for 62.3% of fungal isolates, and ocular surface disease and previous corneal surgery were the most frequent risk factors (Ting et al., 2021). In Galicia, a humid temperate region of north-western Spain with an ageing and largely rural population, *Candida* species caused 55.8% of 77 cases at a median age of 68 years, and patients with *Candida* infection were older than those with mould infection (Lamas-Francis et al., 2024); in our series, the median age was identical in the mould and yeast groups. Mould predominance in temperate Europe has mainly been described in contact lens wearers: in a Swiss tertiary centre, moulds were 1.8 times more common than yeasts, but the median age was 38 years and 74.8% of patients wore contact lenses (Bajka et al., 2024), consistent with the contact lens-associated shift reported from London (Ong et al., 2016). In neighbouring Greece, *Fusarium* species accounted for 61.8% of isolates in a 16-year nationwide survey of filamentous fungal keratitis, with plant-related corneal injury (42.8%) and soft contact lens use (31.4%) as the main risk factors; *F. solani* was mainly associated with trauma, whereas *Fusarium verticillioides* (Sacc.) Nirenberg and *F. proliferatum* were associated with soft contact lens wear (Mpakosi et al., 2022). Contact lens history was not available in our series, including for the 37-year-old woman with *F. proliferatum* keratitis.

Laboratory-based regional inventories are important because national burden estimates remain uncertain. The model of Hilmioğlu-Polat et al. (2019), based on the Leading International Fungal Education (LIFE) methodology, estimated 26,671 fungal keratitis cases per year in Türkiye (33/100,000) by assuming that 22.3% of microbial keratitis is fungal, a proportion taken from the single-centre İzmir series of 1990–2005 (Yilmaz et al., 2007). The global review by Brown et al. (2021), however, displayed a national rate of 0.12/100,000 for Türkiye and used this rate for its Western Asia estimate, attributing it to the same national source. The approximately 275-fold difference between these two modelled figures illustrates how few primary data on local etiology are available.

The predominance of *Fusarium* has direct therapeutic implications. In the Mycotic Ulcer Treatment Trial, topical natamycin was associated with better clinical and microbiological outcomes than voriconazole in filamentous fungal keratitis, with much of the difference attributable to *Fusarium* cases (Prajna et al., 2013). Topical natamycin has repeatedly been reported as unavailable in Türkiye (Hilmioğlu-Polat et al., 2019; Harbiyeli et al., 2022; Öner et al., 2023), and Harbiyeli et al. (2022) identified its absence as an important barrier to the treatment of *Fusarium* keratitis. Because yeasts also accounted for 30% of our isolates, rapid laboratory distinction between moulds and yeasts remains important for guiding initial therapy.

Species-level identification also highlighted the clinical relevance of this small series. Eight of the ten isolates belong to taxa included in the WHO fungal priority pathogens list: *C. albicans* in the critical group, *Fusarium* spp. and *C. parapsilosis* in the high-priority group, and *S. prolificans* (listed as *L. prolificans*) in the medium-priority group (World Health Organization [WHO], 2022). In that prioritisation, *S. prolificans* ranked first for research and development need because of the lack of effective treatment options, although its rarity lowered its perceived public health importance (WHO, 2022). In a national systematic review, the eye was the most frequently involved site among 165 rare mould infections reported from Türkiye (44 cases, 26.7%), mostly due to *Fusarium* spp. (28 of 44) and *Scedosporium*/*Pseudallescheria* spp. (7 of 44), and no ocular infection due to *S. prolificans* or *A. pullulans* was included (Gülmez, 2022); a subsequent Turkish report of *Scedosporium* keratitis likewise involved *Scedosporium apiospermum* (Sacc.) Sacc. ex Castell. & Chalm. (Erkan Pota et al., 2022). Elsewhere, *S. prolificans* has recently been described as an emerging ocular pathogen: at a North Indian centre it was identified in three of eight *Scedosporium*/*Lomentospora* ocular infections over nine months, and two keratitis cases presented with complete corneal melt (Saigal et al., 2025). Its recovery from a corneal scraping should therefore prompt early communication with the ophthalmology team and consideration of specialist management.

A. pullulans is a ubiquitous saprophyte, found particularly in humid environments and commonly isolated from plant debris, soil and wood; it is also a common contaminant in clinical laboratories, and infection is usually not suspected until the fungus is isolated (Suman & Kumar, 2026). Keratitis due to this organism is rare. In a recently reported case without preceding ocular trauma or surgery, a corneal ulcer that had not responded to two months of empirical treatment was diagnosed only when Sabouraud dextrose agar yielded *A. pullulans* after three weeks of incubation, and it healed with topical natamycin and systemic itraconazole (Suman & Kumar, 2026). In Türkiye, *A. pullulans* has been documented as

the agent of a disseminated infection in a renal transplant recipient and has also been isolated from skin/soft tissue and sinus infections (Gülmez, 2022). Its association with humid environments and plant material is compatible with, but does not establish, an environmental source in the humid Eastern Black Sea region.

Melanised fungal keratitis appears to be rarely documented in Türkiye: the national review identified ocular cases due to *Alternaria* and *Fonsecaea* spp. (Gülmez, 2022), and the first keratitis due to *Curvularia lunata* (Wakker) Boedijn from Türkiye was reported in 2023, when ITS sequencing was required because conventional methods cannot reliably separate similar dematiaceous genera (Öner et al., 2023). Direct microscopy of corneal smears may suggest a pigmented fungus but cannot distinguish fungal genera or species (Brown et al., 2021), and *A. pullulans* is reliably differentiated from other dematiaceous fungi by DNA sequencing (Suman & Kumar, 2026). Sequencing and susceptibility testing of such isolates would be particularly informative in Türkiye: the *in vitro* data summarised by Suman and Kumar (2026) indicate activity of amphotericin B, itraconazole and posaconazole but high voriconazole MICs for *A. pullulans*, whereas natamycin is unavailable and fortified topical voriconazole has been used as empirical antifungal therapy (Harbiyeli et al., 2022). Isolation of such organisms from corneal material should therefore be interpreted together with clinical findings, repeat growth and trauma or surgical history rather than dismissed automatically.

This study has several limitations. The number of isolates was small and derived from a single centre, and all patients were inpatients, which may bias the series towards more severe disease. The denominator comprised culture-positive isolates rather than all corneal scrapings or all patients with clinical keratitis. Culture has limited sensitivity for fungal keratitis, reaching at best 73% even in expert hands (Brown et al., 2021), and concurrent bacterial growth may hinder detection of slower-growing fungi (Gülmez, 2022); culture-negative and polymicrobial fungal keratitis may therefore have been under-captured. Slow-growing agents may also be missed if incubation is too short: in the recent *A. pullulans* keratitis case, culture became positive only after three weeks (Suman & Kumar, 2026), and some corneal specimens have required up to 35 days to grow (Brown et al., 2021). Risk factors, contact lens use, treatment and clinical outcomes were not available, antifungal susceptibility testing was not performed, and MALDI-TOF MS identifications were not confirmed by sequencing.

In this ten-year laboratory series from the Eastern Black Sea region of Türkiye, moulds, particularly *Fusarium* species, were the principal agents of keratomycosis in a predominantly older patient population, a profile consistent with earlier Turkish data from the Aegean region and distinct from yeast-predominant series reported from İstanbul and temperate Europe. The identification of rare and difficult-to-treat opportunists such as *S. prolificans* and *A. pullulans*, neither of which was represented among ocular rare mould infections compiled from Türkiye up to 2021, shows that the clinical and scientific value of this series lies in its qualitative diversity. Continuous, long-term surveillance of local fungal etiologies, combined with the systematic collection of risk factor, outcome and susceptibility data, is needed to expand regional epidemiological data, support accurate species identification and guide targeted empirical antifungal therapy.

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Clinical Distribution and Antifungal Susceptibility Profiles of *Pichia kudriavzevii* (formerly *Candida krusei*) Isolates: A Nine-Year Retrospective Evaluation

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Abstract

Pichia kudriavzevii, which was formerly named and is still commonly known as *Candida krusei,* is a rare but well-recognised fungal pathogen classified under the "Medium Priority" category in the World Health Organization (WHO) Fungal Priority Pathogens List due to its intrinsic resistance to fluconazole and its association with high mortality among vulnerable groups. This study aimed to evaluate the clinical distribution and antifungal susceptibility profiles of *P. kudriavzevii* strains isolated from sterile body fluids and bile specimens in our laboratory at a tertiary healthcare facility over a nine-year period.

A total of 35 *P. kudriavzevii* isolates recovered from sterile body fluid and bile specimens between 2017 and 2025 were evaluated. Isolates were identified via conventional methods and Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS, Bruker Daltonics, Germany). Only the first isolate per patient was included. Antifungal susceptibility testing was performed using the Sensititre YeastOne colorimetric microdilution panel, and interpreted according to the recommendations of the Clinical Laboratory Standards Institute. MIC intervals, MIC_{50}, and MIC_{90} values were determined for eight antifungals (anidulafungin, micafungin, caspofungin, flucytosine, posaconazole, voriconazole, itraconazole, amphotericin B).

Among the 35 isolates, the most common specimen type was blood (n=15, 42.9%), followed by peritoneal fluid (n=9, 25.7%), bile (n=8, 22.8%), and pleural fluid (n=3, 8.6%). Echinocandins exhibited potent in vitro activity; micafungin demonstrated the lowest MIC_{90} (0.25 µg/mL), followed by caspofungin (0.5 µg/mL) and anidulafungin (2 µg/mL). Among the azoles tested voriconazole and posaconazole both showed a MIC_{90} of 1 µg/mL, while itraconazole's MIC_{90} was 0.5 µg/mL. Amphotericin B maintained stable activity with MIC_{50} and MIC_{90} values of 1 µg/mL.

Our findings demonstrate that invasive *P. kudriavzevii* infections predominantly manifest as candidemia and intra-abdominal infections. Echinocandins and amphotericin B remain highly effective options in vitro, while voriconazole serves as a reliable alternative azole. Given its WHO priority status, continuous surveillance of susceptibility profiles is essential to detect evolving resistance patterns and to reduce the mortality rate in invasive infections.

Keywords: *Pichia kudriavzevii*, antifungal susceptibility profiles, sterile body fluids, bile

Pichia kudriavzevii (Eski Adıyla *Candida krusei*) İzolatlarının Klinik Dağılımı ve Antifungal Duyarlılık Profilleri: Dokuz Yıllık Retrospektif Değerlendirme

Özet

Eski adı *Candida krusei* (Castell.) Berkhout olan ve bu adla hâlâ yaygın olarak bilinen *Pichia kudriavzevii* Boidin, Pignal & Besson, nadir görülen ancak iyi tanınan bir fungal patojendir. Flukonazole intrinsik dirençli olduğu ve savunmasız hasta gruplarında yüksek mortaliteyle ilişkili bulunduğu için Dünya Sağlık Örgütü (DSÖ) Fungal Öncelikli Patojenler Listesi'nde "Orta Öncelikli" kategoride yer alır. Bu çalışmada, üçüncü basamak bir sağlık kuruluşundaki laboratuvarımızda dokuz yıl boyunca steril vücut sıvıları ve safra örneklerinden izole edilen *P. kudriavzevii* suşlarının klinik dağılımını ve antifungal duyarlılık profillerini değerlendirmeyi amaçladık.

2017–2025 yılları arasında steril vücut sıvısı ve safra örneklerinden üretilen toplam 35 *P. kudriavzevii* izolatı değerlendirilmiştir. İzolatlar konvansiyonel yöntemlerle ve Matriks Destekli Lazer Desorpsiyon İyonizasyon-Uçuş Süresi Kütle Spektrometrisi (MALDI-TOF MS, Bruker Daltonics, Almanya) ile tanımlanmıştır. Her hastadan yalnızca ilk izolat çalışmaya alınmıştır. Antifungal duyarlılık Sensititre YeastOne kolorimetrik mikrodilüsyon paneliyle test edilmiş, sonuçlar Clinical and Laboratory Standards Institute önerilerine göre yorumlanmıştır. Sekiz antifungal ajan (anidulafungin, mikafungin, kaspofungin, flusitozin, posakonazol, vorikonazol, itrakonazol, amfoterisin B) için MIC aralıkları ile MIC_{50} ve MIC_{90} değerleri belirlenmiştir.

35 izolatta en sık örnek türü kan (n=15, %42,9) olmuş; bunu periton sıvısı (n=9, %25,7), safra (n=8, %22,8) ve plevra sıvısı (n=3, %8,6) izlemiştir. Ekinokandinler in vitro güçlü etkinlik göstermiştir; en düşük MIC_{90} değeri mikafungin ve anidulafunginde (0,25 µg/mL), ardından kaspofunginde (0,5 µg/mL) saptanmıştır. Test edilen azollerden vorikonazolün MIC_{90} değeri 1 µg/mL, posakonazol ve itrakonazolün MIC_{90} değeri 0,5 µg/mL bulunmuştur. Amfoterisin B, MIC_{50} ve MIC_{90} değerleri 1 µg/mL olmak üzere etkinliğini kararlı biçimde korumuştur.

Bulgularımız, invaziv *P. kudriavzevii* enfeksiyonlarının çoğunlukla kandidemi ve intraabdominal enfeksiyon olarak ortaya çıktığını göstermektedir. Ekinokandinler ve amfoterisin B in vitro yüksek etkinliğini korurken vorikonazol güvenilir bir alternatif azol olarak öne çıkmaktadır. Tür DSÖ öncelik listesinde yer aldığı için gelişen direnç örüntülerini saptamak ve invaziv enfeksiyonlarda mortaliteyi azaltmak amacıyla duyarlılık profilleri sürekli izlenmelidir.

Anahtar Kelimeler: *Pichia kudriavzevii*, antifungal duyarlılık profilleri, steril vücut sıvıları, safra

Introduction

Pichia kudriavzevii, still widely known by its former name *Candida krusei*, was renamed in the revised medical mycology nomenclature to reflect its phylogenetic position, and the new name also accords with the unusual resistance profile of the species (Borman & Johnson, 2021). It is a rare but well-recognised cause of invasive fungal disease, particularly in patients with haematological or gastric malignancy, neutropenia, underlying gastrointestinal disease or a history of azole exposure. Mortality rates of up to 67% have been reported in adults with candidaemia (Nguyen et al., 2024). Because of its intrinsic resistance to fluconazole and decreased susceptibility to amphotericin B, species-level identification directly determines the appropriateness of antifungal therapy, and the species was included in the medium-priority group of the first fungal priority pathogens list (Nguyen et al., 2024; World Health Organization [WHO], 2022).

The systematic review that informed this list found epidemiological and susceptibility data on *P. kudriavzevii* to be scarce and called for surveillance systems to monitor the incidence and susceptibility of the species (Nguyen et al., 2024). Local data are also needed because susceptibility profiles vary between regions (Chen et al., 2026) and because species-specific interpretive criteria are available for only some of the antifungal agents routinely tested (Clinical and Laboratory Standards Institute [CLSI], 2026). This study aimed to evaluate the clinical distribution, temporal laboratory share, and antifungal susceptibility profiles of *P. kudriavzevii* isolates recovered from sterile body fluids and bile over a nine-year period (2017–2025), using current CLSI interpretive criteria.

Materials and Methods

Study Design and Setting

This retrospective, laboratory-based descriptive study was conducted in the Medical Microbiology Laboratory of Karadeniz Technical University Faculty of Medicine, a tertiary-care university hospital in Trabzon, Türkiye. Laboratory information system records from 2017 to 2025 were reviewed.

Laboratory-Wide Yeast Population

To place the invasive cohort in context, all yeast isolates reported from any clinical specimen during the study period were extracted (25,915 raw records). Records were de-duplicated to one isolate per patient per species, yielding 11,873 isolates from 9,853 patients; this population served as the denominator for the overall, annual and specimen-type-specific shares of *P. kudriavzevii*. Because this analysis retained one isolate per patient per species irrespective of specimen type, its specimen-level counts are not directly comparable with those of the invasive cohort. Both the numerators and the denominators of the specimen-type-specific shares were derived from this population.

Invasive Cohort

Records reported as *P. kudriavzevii* or *C. krusei* were screened (467 de-duplicated isolates). Isolates recovered from blood, peritoneal fluid, pleural fluid and bile were eligible, and only the first eligible isolate per patient was included, giving a final cohort of 35 isolates. Specimen type, requesting clinical unit and patient age group were recorded.

Identification and Antifungal Susceptibility Testing

Isolates were identified by conventional methods and matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics, Germany). Antifungal susceptibility testing was performed with the Sensititre YeastOne colourimetric broth microdilution panel (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. Quality control was performed according to the laboratory's routine procedures using *Candida parapsilosis* (Ashford) Langeron & Talice ATCC 22019 and *P. kudriavzevii* ATCC 6258. Minimum inhibitory concentrations (MICs) of anidulafungin, micafungin, caspofungin, voriconazole, posaconazole, itraconazole, amphotericin B and flucytosine were analysed. Fluconazole was not analysed because *P. kudriavzevii* is intrinsically resistant to this agent (Nguyen et al., 2024).

Interpretation of MIC Values

MICs were interpreted according to CLSI M27M44S, 4th edition (CLSI, 2026). Species-specific clinical breakpoints were applied to anidulafungin, micafungin and caspofungin (susceptible [S] ≤0.25, intermediate [I] 0.5, resistant [R] ≥1 µg/mL) and to voriconazole (S ≤0.5, I 1, R ≥2 µg/mL). For agents without clinical breakpoints, isolates were classified as wild-type (WT) or non-wild-type (NWT) using CLSI epidemiological cutoff values (ECVs): posaconazole WT ≤0.5, itraconazole WT ≤1 and amphotericin B WT ≤2 µg/mL (CLSI, 2022). No interpretive criteria are available for flucytosine, and its MIC distribution was therefore reported descriptively.

Statistical Analysis

All analyses were descriptive. Categorical variables, including specimen type, study period and interpretive category, were presented as counts and percentages. For each antifungal agent, the MIC range, MIC_{50}, MIC_{90} and geometric mean (GM) MIC were determined. MIC_{50} and MIC_{90} were defined as the lowest concentrations inhibiting at least 50% and 90% of isolates, respectively. The percentages of susceptible and wild-type isolates were calculated for agents with CLSI clinical breakpoints and epidemiological cutoff values, respectively. The share of *P. kudriavzevii* among all yeast isolates was calculated overall, by specimen type, for each year and for the periods 2017–2020 and 2021–2025. As the study was designed as a descriptive surveillance analysis, no hypothesis testing was performed;

differences between periods and specimen types were reported descriptively, without statistical inference.

Results

Share of *P. kudriavzevii* Among Laboratory Yeast Isolates

Of the 11,873 yeast isolates, *P. kudriavzevii* accounted for 467 (3.9%) and was the sixth most frequent species after *Candida albicans* (C.P. Robin) Berkhout, *Kluyveromyces marxianus* (E.C. Hansen) Van der Walt, *Nakaseomyces glabratus* (H.W. Anderson) Sugita & M. Takash. (formerly *C. glabrata*), *Candida tropicalis* (Castell.) Berkhout and *C. parapsilosis*. The annual share of *P. kudriavzevii* ranged from 1.9% in 2017 to 6.3% in 2024 and increased from 2.6% in 2017–2020 to 4.8% in 2021–2025 (Figure 1a). The share differed by specimen type: it was highest in bile (10.7%) and pleural fluid (10.3%), followed by peritoneal fluid (6.9%), and was lowest in blood (2.9%) (Figure 2).

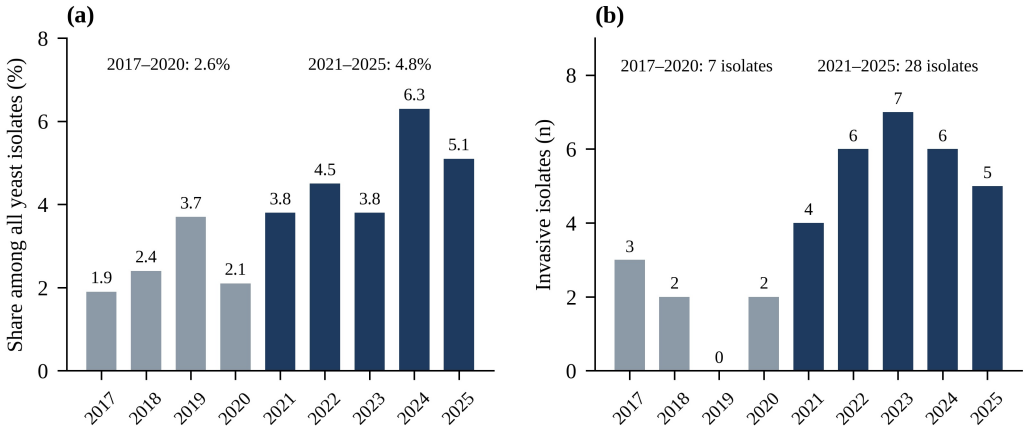


Figure 1. Temporal distribution of *P. kudriavzevii*, 2017–2025. (a) Annual share of *P. kudriavzevii* among all yeast isolates in the laboratory (one isolate per patient per species; n = 11,873). (b) Annual number of invasive isolates included in the study cohort (n = 35); Grey bars, 2017–2020; dark bars, 2021–2025.

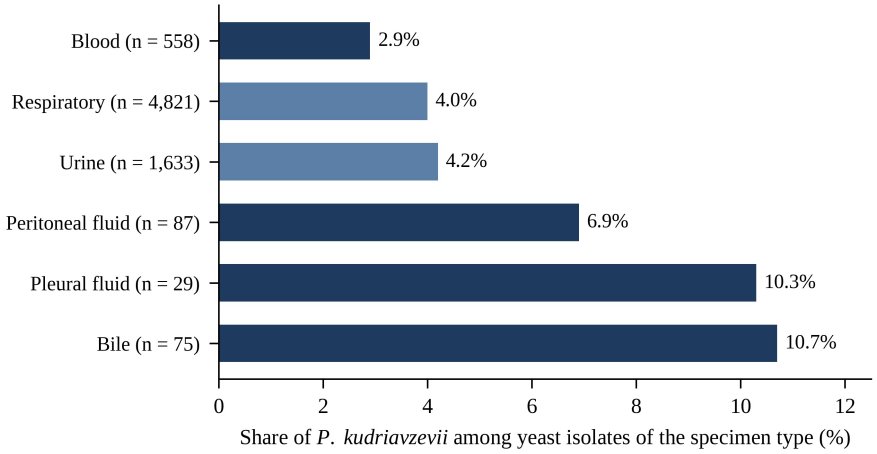


Figure 2. Share of *P. kudriavzevii* among yeast isolates by specimen type. Numerators and denominators were both derived from the de-duplicated laboratory-wide population, in which each patient contributes one isolate per species. Numbers in parentheses indicate the total number of yeast isolates recovered from each specimen type.

Characteristics of the Invasive Cohort

The characteristics of the 35 patients are summarised in Table 1. Blood was the most frequent specimen (15/35, 42.9%), followed by peritoneal fluid (9/35, 25.7%), bile (8/35, 22.8%) and pleural fluid (3/35, 8.6%); peritoneal fluid and bile together accounted for 17 isolates (48.6%). Isolates were most frequently obtained from intensive care units (11, 31.4%) and surgery/gastroenterology units (11, 31.4%), followed by oncology/haematology units (9, 25.7%). Patients aged 18–64 years (14, 40.0%) and ≥65 years (13, 37.1%) predominated, and eight patients (22.9%) were younger than 18 years. The number of invasive isolates increased from 7 in 2017–2020 to 28 in 2021–2025 (Figure 1b).

Table 1. Specimen, clinical unit, age and period distribution of patients with invasive *P. kudriavzevii* isolates (n = 35)

Characteristic	n	%
Specimen type		
Blood	15	42.9
Peritoneal fluid	9	25.7
Bile	8	22.8
Pleural fluid	3	8.6
Clinical unit		
Intensive care unit	11	31.4
Surgery/gastroenterology	11	31.4
Oncology/haematology	9	25.7
Other	4	11.4
Age group (years)		
<18	8	22.9
18–64	14	40.0
≥65	13	37.1
Study period		
2017–2020	7	20.0
2021–2025	28	80.0

Antifungal Susceptibility

MIC distributions and interpretive results are shown in Figure 3 and Table 2. Echinocandins showed the lowest MICs, with MIC_{50}/MIC_{90} values of 0.12/0.25 µg/mL for both anidulafungin and micafungin and 0.25/0.5 µg/mL for caspofungin. Susceptibility rates were 94.3% for anidulafungin (two intermediate and no resistant isolates) and 97.1% for micafungin (one resistant isolate). By contrast, only 21 isolates (60.0%) were categorised as susceptible to caspofungin; of the 14 non-susceptible isolates, 13 had an MIC of 0.5 µg/mL, one dilution above the susceptible breakpoint, and one had an MIC of 1 µg/mL. All caspofungin non-susceptible isolates were susceptible to anidulafungin and micafungin.

For voriconazole, MIC_{50}/MIC_{90} values were 0.5/1 µg/mL; 30 isolates (85.7%) were susceptible, four (11.4%) intermediate and one (2.9%) resistant. Posaconazole and itraconazole both had MIC_{50}/MIC_{90} values of 0.25/0.5 µg/mL; all isolates were WT for itraconazole, whereas three (8.6%) were NWT for

posaconazole. Amphotericin B MICs clustered at 1 µg/mL (25/35, 71.4%; MIC_{50}/MIC_{90} 1/1 µg/mL), and one isolate (2.9%) with an MIC of 4 µg/mL was NWT. Flucytosine MICs formed a narrow high band, with 32 isolates (91.4%) at 8–16 µg/mL (MIC_{50}/MIC_{90} 16/16 µg/mL), one at 32 µg/mL and two at ≤0.06 µg/mL.

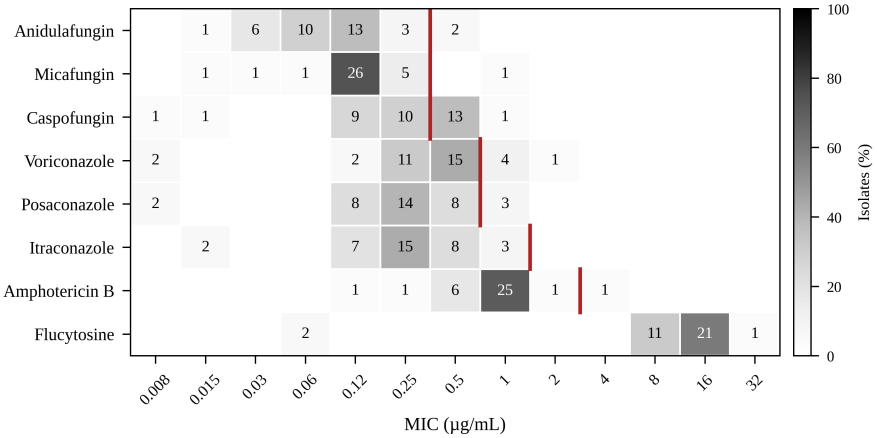


Figure 3. MIC distributions of eight antifungal agents for 35 invasive *P. kudriavzevii* isolates. Cell values indicate the number of isolates and shading indicates the percentage of isolates. Red vertical lines mark the upper limit of the susceptible category (CLSI clinical breakpoint) for echinocandins and voriconazole and the upper limit of the wild-type population (CLSI ECV) for posaconazole, itraconazole and amphotericin B. Values in the lowest column of each agent include off-scale results (≤).

Table 2. In vitro activity and CLSI-based interpretation of eight antifungal agents against invasive *P. kudriavzevii* isolates (n = 35)

Antifungal agent	MIC range (µg/mL)	MIC_{50}/MIC_{90} (µg/mL)	GM MIC	Interpretive criterion (µg/mL)	Category, n (%)
Anidulafungin	0.015–0.5	0.12/0.25	0.09	CBP: S ≤0.25; I 0.5; R ≥1	S 33 (94.3) I 2 (5.7) R 0
Micafungin	0.015–1	0.12/0.25	0.13	CBP: S ≤0.25; I 0.5; R ≥1	S 34 (97.1) I 0 R 1 (2.9)
Caspofungin	≤0.008–1	0.25/0.5	0.24	CBP: S ≤0.25; I 0.5; R ≥1	S 21 (60.0) I 13 (37.1) R 1 (2.9)
Voriconazole	≤0.008–2	0.5/1	0.33	CBP: S ≤0.5; I 1; R ≥2	S 30 (85.7) I 4 (11.4) R 1 (2.9)
Posaconazole	≤0.008–1	0.25/0.5	0.23	ECV: WT ≤0.5	WT 32 (91.4) NWT 3 (8.6)
Itraconazole	0.015–1	0.25/0.5	0.25	ECV: WT ≤1	WT 35 (100) NWT 0
Amphotericin B	≤0.12–4	1/1	0.85	ECV: WT ≤2	WT 34 (97.1) NWT 1 (2.9)
Flucytosine	≤0.06–32	16/16	9.56	None available	8–16: 32 (91.4) 32: 1 ≤0.06: 2

CBP, clinical breakpoint (CLSI M27M44S, 4th ed.); ECV, epidemiological cutoff value (CLSI M57S); GM, geometric mean; S/I/R, susceptible/intermediate/resistant; WT/NWT, wild-type/non-wild-type. MIC_{50} and MIC_{90} describe the MIC distribution and are not susceptibility percentages.

Discussion

This nine-year laboratory-based analysis shows that invasive *P. kudriavzevii* isolates in our setting have an in vitro susceptibility profile largely consistent with published data, whereas their epidemiological pattern differs from that described in the literature in two respects: the share of the species among yeast isolates increased over time, and intra-abdominal and biliary specimens accounted for almost half of the invasive cohort.

The share of *P. kudriavzevii* among yeast isolates from blood (2.9%) is comparable to the 2.1–2.6% reported in global surveillance programmes and lies within the 1–10.8% range described for adult candidaemia in the WHO systematic review (Nguyen et al., 2024); the overall laboratory share (3.9%) is of similar magnitude. The blood share is also close to the 2.6% (52/1,991) share of *C. krusei* among candidaemia isolates in a 12-centre study from Türkiye covering 1997–2017 (Arikan-Akdagli et al., 2019). The temporal pattern, however, differs. The systematic review, which covered studies published between 2011 and 2021, described a generally low and stable incidence, with stable rates of 0–6% in Japan, Denmark, Canada and Saudi Arabia, decreasing rates in France and Israel, and an increase only in India (from 5.6% in 2009–2013 to 9.3% in 2014–2018), which was attributed to high hospital occupancy and difficulties in infection control (Nguyen et al., 2024). In our laboratory, the share rose from 2.6% in 2017–2020 to 4.8% in 2021–2025, and the number of invasive isolates increased from 7 to 28. Because the numerator and denominator were derived from the same database with the same de-duplication rule, this increase is unlikely to be explained by growth in laboratory workload alone. Nevertheless, changes in case-mix, antifungal exposure and identification practices were not assessed, the increase was not tested statistically, and no trend data from Türkiye were reported in the review; the observation therefore requires confirmation by multicentre surveillance.

The specimen distribution was the most distinctive feature of this series. Although blood was the single most common source, the share of *P. kudriavzevii* among yeast isolates was about 3.7-fold higher in bile and 2.4-fold higher in peritoneal fluid than in blood. In a retrospective study of intra-abdominal candidiasis, *C. krusei* represented 3% of 180 *Candida* isolates (Vergidis et al., 2016), and in a prospective multicentre study of patients undergoing endoscopic retrograde cholangiopancreatography, it accounted for 2.6% of fungal species isolated from bile (Lenz et al., 2014). In a recent series of 203 isolates from Shanghai, the species was recovered mainly from sputum and urine (Chen et al., 2026). Underlying gastrointestinal disease and gastric malignancy have been identified as risk factors for *P. kudriavzevii* infection (Nguyen et al., 2024), which is consistent with the substantial proportion of isolates from surgery/gastroenterology units in our cohort. Nosocomial clustering must also be considered, since transmission via the hands of healthcare workers and the hospital environment has been described (Nguyen et al., 2024). Short tandem repeat genotyping of 35 bloodstream *C. krusei* isolates from Türkiye revealed unique genotypes without evidence of clonal transmission (Karakoyun et al., 2024); as our isolates were not genotyped, we cannot determine whether the biliary and peritoneal predominance reflects independent infections or the spread of related strains. In addition, the denominators for bile (n = 75) and pleural fluid (n = 29) were small, making these proportions imprecise, and clinical data distinguishing infection from colonisation were not available.

With respect to susceptibility, resistance rates for all agents with species-specific clinical breakpoints were within the 0–5% range reported for echinocandins and azoles other than fluconazole in the WHO systematic review (Nguyen et al., 2024): no isolate was resistant to anidulafungin, and one isolate each (2.9%) was resistant to micafungin, caspofungin and voriconazole. Likewise, no resistant or NWT isolates were detected among bloodstream isolates in the Turkish genotyping study (Karakoyun et al., 2024). The markedly lower caspofungin susceptibility rate (60.0%) should be interpreted with caution. Three of 16 studies in the systematic review reported caspofungin NWT rates of 30–67%, and the review notes that caspofungin MIC testing is considered unreliable by both CLSI and EUCAST because of interlaboratory variability, with EUCAST recommending anidulafungin or micafungin as markers of echinocandin resistance (Nguyen et al., 2024). In our series, 13 of the 14 caspofungin non-susceptible isolates had an MIC only one dilution above the susceptible breakpoint, while anidulafungin and micafungin showed lower and more homogeneous MIC distributions. These findings suggest a shift in categorical interpretation caused by MIC variability around the breakpoint rather than true echinocandin resistance, although *FKS* sequencing would be needed to exclude resistance mechanisms.

For voriconazole, the resistance rate was low, but four isolates (11.4%) were intermediate. A Sensititre YeastOne-based series from Shanghai reported a voriconazole resistance rate of 9.4% with an increasing trend, and 39.4% of its isolates were NWT for amphotericin B and posaconazole (Chen et al., 2026), illustrating the geographical variability of susceptibility profiles. In our cohort, three isolates (8.6%) were NWT for posaconazole, slightly above the 0–5.6% range reported by most studies of azoles other than fluconazole (Nguyen et al., 2024) and above the 1.9% reported in the Turkish multicentre study (Arikan-Akdagli et al., 2019); because each isolate represents 2.9% of this small cohort, this finding should be monitored rather than over-interpreted. For amphotericin B, 97.1% of isolates were WT, in line with the predominantly wild-type distributions reported in most studies, whereas higher rates (12.9% and 40%) have been described in India and Iran, where amphotericin B is widely used (Nguyen et al., 2024).

Interpretation of the MIC data requires attention to the available criteria. Species-specific clinical breakpoints were available for only four of the eight agents analysed, three agents could be categorised only as WT or NWT, and no interpretive criteria were available for flucytosine (CLSI, 2022, 2026). The innate resistance of *P. kudriavzevii* to fluconazole and flucytosine is regarded as a feature shared by many *Pichia* species (Borman & Johnson, 2021); the narrow high flucytosine band (8–16 µg/mL in 91.4% of isolates) should therefore be read as a species-level characteristic rather than as acquired resistance. It should also be noted that MIC_{50} and MIC_{90} values summarise MIC distributions and should not be reported as susceptibility percentages. For routine practice, reporting MIC values together with the relevant interpretive framework (clinical breakpoint, ECV or none) and prioritising rapid species-level identification are recommended, because rapid identification of *P. kudriavzevii* enables fluconazole to be avoided and appropriate antifungal therapy to be initiated promptly (Nguyen et al., 2024).

This study has limitations. It was a single-centre, retrospective, laboratory-based study with a small number of invasive isolates, and clinical data such as underlying disease, prior antifungal exposure, the

distinction between infection and colonisation, and outcomes were not analysed. Susceptibility testing was performed with a commercial colourimetric method rather than the CLSI reference broth microdilution method on which clinical breakpoints and ECVs are based. Isolates were not genotyped, resistance mechanisms were not investigated, and possible changes in identification practices over the nine-year period may have influenced the temporal trend.

In conclusion, invasive *P. kudriavzevii* isolates in our setting were mainly associated with candidaemia and intra-abdominal or biliary specimens. Anidulafungin, micafungin, voriconazole and amphotericin B retained good in vitro activity, and the low caspofungin susceptibility rate appears to reflect MIC variability near the breakpoint rather than true echinocandin resistance. The main surveillance signal of this series is the increasing local share of the species and its frequent recovery from bile and peritoneal fluid rather than rising resistance. Continuous species-level surveillance, periodic analysis of local MIC distributions and molecular typing of clustered isolates are recommended.

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In Vitro Evaluation of the Antifungal Activity of Different Lactoferrin Preparations Against *Candida albicans*

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Abstract

Lactoferrin is a multifunctional iron-binding glycoprotein that has been reported to exhibit antimicrobial activity against a wide range of microorganisms. However, its antifungal efficacy may vary depending on its source, structural integrity, and formulation. This study aimed to evaluate and compare the in vitro antifungal activity of different lactoferrin preparations against *Candida albicans* ATCC 10231 using the disk diffusion method. Recombinant human lactoferrin (Sigma-Aldrich, L1294), bovine lactoferrin (Sigma-Aldrich, L4765), simulated gastric fluid-derived peptides of recombinant human and bovine lactoferrin, a commercially available lactoferrin-containing pH-balancing formulation (Enzymilk® Sensitive Protection pH Balancer), and a commercially available lactoferrin dietary supplement (Valfoo® Lactoferrin) were tested. Lactoferrin-derived peptides were generated by pepsin digestion in simulated gastric fluid at 37°C for 120 min, verified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and quantified using the Qubit Protein Assay. A 0.5 McFarland suspension of *C. albicans* ATCC 10231 was uniformly inoculated onto Sabouraud Dextrose Agar plates. Sterile blank disks were loaded with 25 µL of each preparation at concentrations of 1000, 500, 250, 125, and 62.5 µg/mL, and each concentration was tested in duplicate. Nystatin (1000 µg/mL) and blank disks served as the positive and negative controls, respectively. After incubation at 37°C for 24 h, inhibition zone diameters were measured. None of the tested lactoferrin preparations, including recombinant human lactoferrin, bovine lactoferrin, their simulated gastric fluid-derived peptides, the commercial pH-balancing formulation, or the dietary supplement, produced inhibition zones beyond the disk diameter at any concentration tested (62.5–1000 µg/mL). In contrast, the positive control, nystatin, produced a mean inhibition zone diameter of 19.7 ± 1.2 mm. These findings indicate that, under the conditions of the disk diffusion assay, the tested lactoferrin preparations did not exhibit detectable antifungal activity against *C. albicans* ATCC 10231. The results suggest that these preparations are ineffective in inhibiting fungal growth under the experimental conditions employed and highlight the importance of evaluating lactoferrin using alternative antifungal susceptibility methods that may better reflect its biological activity.

Keywords: Antifungal agents, lactoferrin, *Candida albicans*, disk diffusion, antimicrobial tests

Farklı Laktoferrin Preparatlarının *Candida albicans*'a Karşı Antifungal Etkinliğinin *İn Vitro* Olarak Değerlendirilmesi

Laktoferrin, çok çeşitli mikroorganizmalara karşı antimikrobiyal aktivite gösterdiği bildirilen, çok işlevli ve demir bağlayan bir glikoproteindir. Ancak antifungal etkinliği; kaynağına, yapısal bütünlüğüne ve formülasyonuna bağlı olarak değişiklik gösterebilir. Bu çalışma, disk difüzyon yöntemi kullanılarak farklı laktoferrin preparatlarının *Candida albicans* ATCC 10231'e karşı *in vitro* antifungal aktivitelerinin değerlendirilmesini ve karşılaştırılmasını amaçlamıştır. Çalışmada rekombinant insan laktoferrini (Sigma-Aldrich, L1294), sığır laktoferrini (Sigma-Aldrich, L4765), rekombinant insan ve sığır laktoferrininin yapay mide sıvısı kaynaklı peptitleri, piyasada mevcut laktoferrin içerikli pH dengeleyici bir formülasyon (Enzymilk® Sensitive Protection pH Balancer) ve piyasada mevcut bir laktoferrin gıda takviyesi (Valfoo® Lactoferrin) test edilmiştir. Laktoferrin kaynaklı peptitler, 37°C'de 120 dakika boyunca yapay mide sıvısı içinde pepsin sindirimi yoluyla elde edilmiş, sodyum dodesil sülfat-poliakrilamid jel elektroforezi ile doğrulanmış ve Qubit Protein Analizi kullanılarak miktarları belirlenmiştir. *C. albicans* ATCC 10231'in 0,5 McFarland yoğunluğundaki süspansiyonu, Sabouraud Dekstroz Agar plaklarına homojen bir şekilde ekilmiştir. Steril boş disklere, 1000, 500, 250, 125 ve 62,5 µg/mL konsantrasyonlarında hazırlanan her bir preparattan 25 µL yüklenmiş ve her konsantrasyon iki tekrarlı olarak test edilmiştir. Nistatin (1000 µg/mL) ve boş diskler sırasıyla pozitif ve negatif kontroller olarak kullanılmıştır. 37°C'de 24 saatlik inkübasyonun ardından inhibisyon zonu çapları ölçülmüştür. Rekombinant insan laktoferrini, sığır laktoferrini, bunların yapay mide sıvısı kaynaklı peptitleri, ticari pH dengeleyici formülasyon veya gıda takviyesi dahil olmak üzere test edilen laktoferrin preparatlarının hiçbiri, incelenen konsantrasyon aralığında (62,5–1000 µg/mL) disk çapını aşan bir inhibisyon zonu oluşturmamıştır. Buna karşılık, pozitif kontrol olan nistatin, 19,7 ± 1,2 mm'lik ortalama bir inhibisyon zonu çapı oluşturmuştur. Bu bulgular, disk difüzyon testi koşulları altında, test edilen laktoferrin preparatlarının *C. albicans* ATCC 10231'e karşı saptanabilir bir antifungal aktivite göstermediğini ortaya koymaktadır. Sonuçlar, söz konusu preparatların uygulanan deneysel koşullar altında fungal büyümeyi inhibe etmede etkisiz olduğunu göstermekte ve laktoferrinin biyolojik aktivitesini daha iyi yansıtabilecek alternatif antifungal duyarlılık yöntemleri kullanılarak değerlendirilmesinin önemini vurgulamaktadır.

Anahtar Kelimeler: Antifungal ajanlar, lactoferrin, *Candida albicans*, disk difüzyon, antimikrobiyal testleri

Introduction

Oral candidiasis is an opportunistic infection of the oral cavity caused by overgrowth of Candida species and can manifest with symptoms such as tongue pain, a burning sensation, and halitosis. Risk factors for oral candidiasis include prolonged antibiotic use, smoking, reduced salivary flow, poor oral hygiene, malnutrition, chemotherapy, and advanced age. Although several *Candida* species can cause oral candidiasis, *Candida albicans* is the most common causative species (Keyvanfar et al., 2024). Oral candidiasis may have clinical implications beyond the oral infection itself. In addition to potentially leading to systemic complications such as aspiration pneumonia, oral candidiasis, particularly its chronic hyperplastic form, has also been associated with potentially malignant oral lesions such as leukoplakia and the development of oral squamous cell carcinoma (Komatsu et al., 2015; Lorenzo-Pouso et al., 2022). Once oral candidiasis develops, it is difficult to eliminate through oral hygiene alone. In addition

to replacing poorly adapted prosthetic restorations, antifungal therapy becomes a fundamental component of treatment. However, antifungal agents have several disadvantages such as drug resistance, adverse reactions, and potential drug-drug interactions (Komatsu et al., 2015; Krupińska & Bogucki, 2024). These limitations highlight the need for alternative antifungal agents with high efficacy, low toxicity, and a reduced risk of resistance development.

Lactoferrin (Lf) is an iron-binding cationic glycoprotein with a molecular weight of approximately 80 kDa. Lf is a protein found in neutrophils and exocrine secretions such as breast milk, tears, nasal secretions, and saliva, where it contributes to mucosal defense against pathogens. Lf has various biological functions and exhibits antimicrobial activity against oral bacteria, fungi, and viruses, as well as immunomodulatory and anti-inflammatory effects (García-Montoya et al., 2012). Lf exhibits antifungal activity against various yeasts and molds, particularly *Candida* species. This activity is primarily mediated by its iron-free form, apo-lactoferrin, which binds Fe^{3+} ions with high affinity, thereby reducing the availability of free iron required for fungal growth. Consequently, it may exert a fungistatic effect (Kirkpatrick et al., 1971). The antifungal effect of Lf is not solely dependent on iron binding. A significant portion of its antifungal activity can occur through direct, iron-independent cellular damage. By interacting directly with the fungal cell surface, Lf can disrupt membrane permeability, leading to loss of cellular contents and proteins, release of potassium ions, altered membrane potential, and intracellular acidification. Cationic peptides generated by the proteolytic digestion of lactoferrin or derived from specific lactoferrin sequences have been reported to exhibit greater antifungal activity and membrane-permeabilizing capacity than the parent protein (Fernandes & Carter, 2017; Viejo-Díaz et al., 2005).

Currently, in addition to recombinant human lactoferrin (rhLf), bovine lactoferrin (bLf), and their peptide derivatives, commercial lactoferrin-containing supplements have also been suggested to support oral health. (Komatsu et al., 2015; Krupińska & Bogucki, 2024). However, studies comparing the antifungal activities of these various preparations under standard in vitro test conditions remain limited. The aim of this study is to evaluate and compare the in vitro antifungal activity of recombinant human lactoferrin, bovine lactoferrin, their peptides derived in simulated gastric fluid (SGF), and commercial lactoferrin preparations against *Candida albicans* ATCC 10231 using the disk diffusion method.

Materials and Methods

Lactoferrin, Lactoferrin-Derived Peptides, and Controls

This study evaluated four lactoferrin-containing preparations from different sources. Recombinant human lactoferrin (rhLf;Sigma-Aldrich, L1294) and bovine lactoferrin (blf;Sigma-Aldrich, L4765) were included as purified lactoferrin preparations. In addition, two commercially available lactoferrin-containing products were evaluated: Enzymilk® Sensitive Protection pH Balancer, a pH-balancing oral formulation, and Valfoo® Lactoferrin, a dietary supplement.

For the antifungal assays, each lactoferrin preparation was prepared under sterile conditions at an initial concentration of 1000 µg/mL using sterile water. The lactoferrin preparations were freshly prepared and

stored at +4°C until use. Nystatin (Carl Roth GmbH+ Co. KG, Karlsruhe, Germany; Cat. No. 0241.1) was dissolved in dimethyl sulfoxide to obtain a final concentration of 1000 µg/mL and was used as a positive antifungal control. Sterile empty disks were used as the negative control.

rhLf and bLf were subjected to in vitro gastric digestion to generate lactoferrin-derived peptides. The *in vitro* gastric digestion procedure was performed based on a previously described method (Gallier et al., 2012) with modifications. Simulated gastric fluid was prepared by adding 140 µL HCl, 40 mg NaCl, and 64 mg pepsin to 15 mL of distilled water (Pharmacopeia, 2000). The solution was mixed until homogeneous; the pH was adjusted to approximately 1.2, and the final volume was brought to 20 mL with distilled water. Prior to digestion, the SGF was pre-incubated at 37°C for 5 min with agitation at 95 rpm. Subsequently, 2 mL of each lactoferrin preparation was mixed with 8 mL of SGF and incubated at 37°C for 120 min with agitation at 95 rpm. At the end of the incubation period, the digestion reaction was terminated by increasing the pH with 1 M Na_2CO_3.

Following digestion, the samples were subjected to sequential ultrafiltration. The samples were first centrifuged using 15-mL centrifugal filter units with a 30-kDa molecular weight cut-off (MWCO) membrane, and the retentate remaining above the membrane was collected. The collected filtrate was then transferred to 3-kDa MWCO centrifugal filter units and centrifuged for 15 min to separate the peptide fraction from digestion-medium components; the retentate remaining above the membrane was collected. Successful peptide cleavage and formation were confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The collected peptide fractions were concentrated by SpeedVac evaporation, quantified using the Qubit Protein Assay (Thermo Fisher Scientific), and subsequently adjusted to a final concentration of 1000 µg/mL.

Fungal Strain, Inoculum Preparation and Antifungal Activity Assessment via Disk Diffusion Assay

Candida albicans ATCC 10231 was used as the fungal indicator. Fresh subcultures were prepared on Sabouraud Dextrose Agar (SDA) plates and incubated at 37°C for 24 hours prior to testing. Fungal cell suspensions were prepared in sterile physiological saline and adjusted to match the 0.5 McFarland turbidity standard. The standardized suspension was homogeneously inoculated onto SDA plates using sterile swabs.

The antifungal activities of all lactoferrin preparations and peptides were evaluated using the disk diffusion method on SDA. Serial two-fold dilutions of each preparation were prepared to obtain final concentrations of 1000, 500, 250, 125, and 62.5 µg/mL. We added 25 µL from each concentration to sterile blank paper disks. All tests were performed in duplicate. The plates were incubated at 37°C for 24 hours, after which the inhibition zone diameters were measured in millimeters (mm).

Results

None of the evaluated lactoferrin preparations, rhLf and bLf digested peptides, or commercial products exhibited a detectable zone of inhibition exceeding the disk diameter across all tested concentrations

(62.5, 125, 250, 500, and 1000 µg/mL). Likewise, the negative control produced no zone of inhibition (<5 mm).

In contrast, the positive control agent, Nystatin (1000 µg/mL), produced a clear and distinct mean inhibition zone diameter of 19.7 ± 1.2 mm, confirming the validity of the assay system and the susceptibility of the test organism.

Discussion

Lactoferrin is a potential agent for preventing and treating oral candidiasis because of its antifungal, immunomodulatory, anti-inflammatory, and antimicrobial properties, as well as its physiological presence in the oral cavity (Krupińska & Bogucki, 2024). In the present study, various lactoferrin preparations, including recombinant human lactoferrin (rhLF), bovine lactoferrin (bLF), peptides derived from SGF-digested rhLF and bLF, and commercial products (Enzymilk® and Valfoo®), did not exhibit detectable antifungal activity against C. albicans ATCC 10231 at concentrations ranging from 62.5 to 1000 µg/mL in the disk diffusion assay.

Lf molecules from different species are highly homologous. However, their antifungal activity may vary due to minor structural differences. Furthermore, Lf preparations with similar purity levels may vary significantly among manufacturers, ranging from highly active to inactive against the same test organism (Fernandes & Carter, 2017; Fernandes et al., 2020). It has been reported that the antifungal activity of lactoferrin can be influenced by its iron saturation status and that iron-saturated holo-lactoferrin may exhibit lower or undetectable antifungal activity compared to iron-depleted apo-lactoferrin (Apo-Lf) (Fernandes & Carter, 2017; Kuipers et al., 1999). The iron saturation levels of the commercial lactoferrin preparations used in this study were not determined. Therefore, the high iron saturation of these preparations may be one of the factors contributing to the lack of observed antifungal activity in our study.

In a study, the antifungal activities of Lf, Apo-Lf, and oligosaccharides derived from various milk sources against fluconazole-resistant *C. albicans* ATCC 10231 were evaluated using the disk diffusion method on SDA and Mueller-Hinton agar supplemented with 2% glucose and methylene blue (MH-GMB). It was reported that, on SDA, the various lactoferrin preparations produced inhibition zones ranging from 16.67 to 23 mm, with the greatest inhibition observed for bovine and goat lactoferrin, whereas Apo-lf and oligosaccharides did not produce inhibition zones. In contrast, no inhibition zones were observed for any evaluated preparation on MH-GMB (Stella et al., 2022). In a study by Rastogi et al., fragments generated by the trypsin digestion of bovine lactoferrin were purified via cation-exchange and gel-filtration chromatography and subsequently converted to their apo-forms by removing iron through dialysis against citric acid. The antifungal activity of these Apo-Lf fragments and Apo-Lf against C. albicans ATCC 90028 was evaluated using the disk diffusion method on Yeast Extract Peptone Dextrose agar and it has been reported that all fragments exhibited higher antifungal activity than natural lactoferrin (Rastogi et al., 2014). Findings from previous studies suggest that the antifungal activity of lactoferrin in disk diffusion tests can be influenced by both the lactoferrin preparation and the test medium. The failure to observe zones of inhibition for lactoferrin and lactoferrin-derived

peptides in the disk diffusion test may be attributed to the physicochemical properties of the solid agar medium. Lactoferrin is a cationic glycoprotein with a molecular weight of approximately 80 kDa, and a significant proportion of the peptides generated through its proteolytic digestion also exhibit cationic characteristics (Viejo-Díaz et al., 2005). Negatively charged groups within the agar matrix, particularly sulfate residues, may interact electrostatically with cationic antimicrobial molecules, thereby limiting their diffusion through the medium (Steinberg & Lehrer, 1997; Twomey et al., 2021).

Overall, the absence of an inhibition zone in the disk diffusion assay may not necessarily indicate a complete lack of antifungal activity in the lactoferrin preparations. Therefore, future studies should determine the iron saturation levels of the preparations and assess their antifungal activity using methods independent of agar diffusion, such as broth microdilution.

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Utilizing Food Industry Waste Through Fungi: Green Biotechnology Applications

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Abstract

Globally, the food industry produces enormous quantities of biodegradable waste with high organic loads, leading to environmental pollution. Traditional methods of waste management are often insufficient. Within the scope of green biotechnology applications, fungi stand out as powerful bioconverters that transform this waste into value-added products. This review presents a comprehensive literature search on the utilization of food waste through fungal fermentation processes, the fungi used, the bioprocesses applied, and the tangible products obtained (single-cell proteins, enzymes, biofuels, and bioactive compounds). Studies show that converting wastes such as bread scraps, olive pomace, sugar beet pulp, and brewer's pulp into fungal biomass can close protein gaps, reduce environmental pollution, and contribute to the circular bioeconomy.

Keywords: Food waste, fungi, green biotechnology, biovalorization, circular bioeconomy

Gıda Sektörü Atıklarının Funguslar Aracılığı ile Değerlendirilmesi: Yeşil Biyoteknoloji Uygulamaları

Özet

Dünya genelinde gıda sektörü, yüksek organik yük içeren ve çevre kirliliğine yol açan muazzam miktarlarda biyobozunur atık üretmektedir. Bu atıkların geleneksel yöntemlerle yönetimi çoğu zaman yetersiz kalmaktadır. Yeşil biyoteknoloji uygulamaları kapsamında funguslar (mantarlar), bu atıkları katma değerli ürünlere dönüştüren güçlü biyodönüştürücüler olarak öne çıkmaktadır. Bu derleme, gıda atıklarının fungal fermentasyon süreçleri aracılığıyla değerlendirilmesini, kullanılan mikroorganizmaları, uygulanan biyoprosesleri ve elde edilen somut ürünleri (tek hücre proteini, enzimler, biyoyakıtlar ve biyoaktif bileşikler) kapsamlı bir literatür taraması ile sunmaktadır. Yapılan çalışmalar ekmek artıkları, zeytin küspesi, şeker pancarı posası ve bira posası gibi atıkların fungal biyokütleye dönüştürülmesiyle protein açığının kapatılabileceğini, çevresel kirliliğin azaltılabileceğini ve döngüsel biyoekonomiye katkı sağlanabileceğini göstermektedir.

Anahtar kelimeler: Gıda atığı, Mantarlar, Yeşil biyoteknoloji, Biyodeğerlendirme, Döngüsel biyoekonomi.

Introduction

The food industry is a major source of waste that is biodegradable but difficult to manage due to its high organic load. Traditional methods, such as the direct discharge of this waste into the environment or incineration, are not sustainable. Green biotechnology offers a circular approach to this problem by treating waste as a raw material. At the heart of this approach are filamentous fungi, yeasts, and macrofungi, which possess the ability to break down lignocellulosic structures and produce a wide variety of secondary metabolites (Khatami et al., 2026; Wikandari et al., 2022).

Fungi possess enzyme systems capable of breaking down complex plant fibers (cellulose, hemicellulose, and lignin) into simpler compounds that they can metabolize (Sumarriva-Bustinza et al., 2025). Thanks to these capabilities, they can convert food industry byproducts into high-protein biomass, industrial enzymes, bioactive compounds, and biofuels (Abd El Aty, 2023; Zaier et al., 2021). This review examines biovalorization processes carried out using fungi and the concrete findings of these processes within the scope of green biotechnology.

Bioconversion Pathways and Fungal-Derived Products

Fungal Bioprocesses and Evaluation Methods

Two main fermentation methods are applied in the biological conversion of food waste: Solid State Fermentation (SSF) and Liquid State Culture.

Solid State Fermentation (SSF): It is preferred especially for agro-industrial wastes and lignocellulosic residues (sugar cane bagasse, wheat bran, etc.) (Abd El Aty, 2023). SSF has advantages such as mimicking the natural habitats of filamentous fungi, being low-cost, producing less wastewater, and providing high enzyme yield (Borkertas et al., 2025). For example, 47 different fungal species isolated from olive oil mill waste in Tunisia were used in the production of industrial enzymes such as lipase, protease, amylase, and cellulase using the SSF method (Zaier et al., 2021). In addition, kitchen wastes are evaluated for laccase production using the SSF method (Nargotra et al., 2024).

Liquid State Fermentation and Bioreactors: It is used under more controlled parameters, especially in the production of bioactive compounds and macrofungi biomass (Pilafidis et al., 2022). The success of scalable systems such as airlift bioreactors in converting bread waste into biomass has been reported (Svensson et al., 2024).

Types of Waste and Microorganisms Used

The literature indicates that a wide variety of food wastes have been evaluated using specific fungal species:

Bread Waste: Bread waste, a global problem, serves as an excellent substrate for species such as *Neurospora intermedia*, *Aspergillus oryzae*, and *Rhizopus delemar* (Kawa-Rygielska et al., 2022; Svensson et al., 2021, 2024). These fungi can consume bread waste without the need for any additional enzymes or nutrients, producing a biomass containing between 27% and 36% protein (Svensson et al., 2021).

Olive Mill Waste: Olive mill waste (OMWW) is used by species such as *Trichoderma virens* and *Aspergillus niger* for enzyme production (Zaier et al., 2021).

Sugar Beet and Agro-Industrial Residues: Sugar beet pulp was fermented with *Rhizopus arrhizus*, increasing its protein content from 53.7 mg/g to 134.11 mg/g (Gaizauskaite & Zadeike, 2025). Similarly, brewer's spent grain (BSG) is processed using *Aspergillus* and *Trichoderma* species to enhance its nutritional value and produce animal feed (Marcus & Fox, 2021).

Enzyme Production Yields

Studies in the literature show that there are significant differences in enzyme production yields depending on the type of fungus and substrate used. Table 1 presents the enzyme yields obtained from selected studies.

Table 1. Enzyme yields obtained from selected fungal bioprocesses.

Strains	Substrate	Enzymes Produced and Yield	Reference
Pleurotus dryinus	Mandarin peels and tree leaves	Laccase (6493 U/mL), Xylanase (71.7 U/mL), Cellulase (47.7 U/mL)	Pilafidis et al., 2022
Trametes ochracea	Olive mill wastewater	Laccase (14,967 IU/L)	Pilafidis et al., 2022
Penicillium fuscoglaucum JAM-1	Rapeseed cake (RSC)	Xylanase (612 U/gds), Endoglucanase (102 U/gds)	Joshi, et al., 2025
Trichoderma virens	Olive mill wastewater	Cellulase (14.55 U/gds), Lipase (33.56 U/gds), Phytase (45.90 U/gds)	Zaier et al., 2021
Aspergillus niger	Olive mill wastewater	Cellulase (9.92 U/gds), Lipase (24.55 U/gds), Phytase (32.55 U/gds)	Zaier et al., 2021
Aspergillus awamori	Bread waste	Glucoamylase (114 U/g), Protease (83.2 U/g)	Kawa-Rygielska, et al., 2022
Trametes versicolor	Brewer's spent grains	Laccase (560 U/L)	Pilafidis et al., 2022
Trichoderma asperellum R	Fruit scrap and green waste	Cellulase Activity (No numerical data specified)	Bulgari et al., 2024

Value-Added Bioproducts and Applications

Products derived from fungal biotransformation processes are critical for a sustainable future.

Alternative Protein Sources and Biomass

The "single cell protein" (SCP) or "mycelium biomass" produced by mushrooms offers an environmentally friendly alternative to animal protein sources (Ohimain, 2025; Sumarriva-Bustinza et al., 2025). Obtaining 19.2 g/L of biomass (containing 45% protein) from bread waste using *Neurospora intermedia* proves the efficiency of this method (Kawa-Rygielska et al., 2022). The richness of the amino acid and fatty acid profiles of these biomasses in terms of nutrition has been confirmed (Svensson et al., 2021).

Industrial Enzymes and Bioactive Compounds

Mushrooms can produce enzymes such as laccase, xylanase, and cellulase, which are cornerstones of green biotechnology, with high yields. The value of 6493 U/mL obtained in laccase production with *P. dryinus* is particularly noteworthy (Pilafidis et al., 2022). In addition, valuable compounds such as vanillic acid and limonoid have been obtained from fruit residues using *Trichoderma asperellum* (Bulgari et al., 2024). Bio-pigments with antioxidant and antimicrobial properties obtained from filamentous fungi also have application potential in the pharmaceutical and food sectors (Filament fungi grown..., 2022).

Biofuels and Innovative Applications

Energy sources such as ethanol (32.2 g/L), biohydrogen and biodiesel can be produced by fungal fermentation of food wastes (Canto-Robertos et al., 2022; Kawa-Rygielska et al., 2022). In addition, in studies pushing the boundaries of biotechnology, it has been observed that chitin/chitosan-rich monofilaments produced from bread wastes accelerate wound healing in medical textiles (Svensson et al., 2024). Fungal residues, on the other hand, provide functional components (β-glucan, phenolics) to improve the mechanical and barrier properties of biodegradable food packaging (Törős et al., 2025).

Conclusion

The utilization of food sector waste through fungi is one of the most successful examples of green biotechnology applications that transform the concept of "waste" into a "resource." Filamentous fungi and macrofungi, thanks to their metabolic flexibility, can convert even the most resistant lignocellulosic wastes into protein-rich biomass, industrial enzymes, and energy sources. Concrete findings show that these processes not only reduce the environmental burden but also offer sustainable solutions to fundamental problems such as global protein demand and energy needs. In the future, widespread adoption of these processes on an industrial scale is vital for strengthening the circular bioeconomy and ensuring food security.

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ORAL PRESENTATIONS

Medical and Veterinary Mycology

Thematic and Methodological Analysis of Postgraduate Theses on *Candidozyma auris* in Türkiye

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Abstract

Candidozyma auris is an emerging opportunistic fungal pathogen of global concern due to its multidrug resistance and potential to cause healthcare-associated outbreaks. This study aimed to evaluate the thematic focus, methodological approaches, and scientific trends of postgraduate theses on *C. auris* conducted within Departments of Medical Microbiology in Türkiye. In this descriptive study, data were retrieved from the National Thesis Center of the Council of Higher Education of the Republic of Türkiye using the keyword "*Candidozyma auris*". Publicly accessible postgraduate theses in Medical Microbiology were included and evaluated by thesis type, year of completion, university, methodological approach, and microbiological research focus. A total of nine theses (seven medical specialty theses and two master's theses) were included. All theses were completed between 2025 and 2026, indicating a recent increase in research interest in *C. auris* in Türkiye. The studies were conducted at İstanbul Medeniyet, Bursa Uludağ, Marmara, Ankara, Akdeniz, Erciyes, Fırat, Gaziantep, and Koç Universities. Research topics primarily focused on species identification, antifungal susceptibility, molecular epidemiology, and virulence characteristics. Studies comparing phenotypic and molecular identification methods demonstrated that automated identification systems may misidentify *C. auris* as a closely related species, whereas CHROMagar *Candida* Plus showed high diagnostic accuracy. Antifungal susceptibility studies compared Sensititre YeastOne, CLSI, and EUCAST methodologies; fluconazole resistance was consistently reported at high levels, while amphotericin B resistance varied depending on the testing method and isolate characteristics. In addition, several theses investigated molecular epidemiology using whole-genome sequencing, and one thesis evaluated the inhibitory activity of chitosan-supported gold nanoparticles against resistant *C. auris* isolates. Postgraduate research on *C. auris* in Türkiye has primarily focused on diagnostic methods, antifungal resistance, and molecular epidemiology. However, the limited number of theses and the concentration of studies within university hospitals indicate that national research capacity in this field remains insufficient. Expanding multicenter, interdisciplinary postgraduate research that incorporates genomic surveillance and infection control strategies will contribute to a better understanding of the resistance mechanisms and transmission dynamics of *C. auris*.

Keywords: *Candidozyma auris*, antifungal resistance, identification, postgraduate theses, bibliometric analysis, Türkiye.

Investigation and comparison the antifungal properties of graphene oxide, silver alumina and silver-alumina graphene oxide against clinical isolates of *Aspergillus*

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Abstract

The worldwide rise in antifungal resistance among *Aspergillus* species represents a significant clinical challenge and highlights the urgent need for innovative antifungal therapies. Therefore, this study aimed to evaluate and compare the antifungal activities of three nanoparticle formulations, including graphene oxide (GO), a silver–alumina nanocomposite (Ag/Al_2O_3), and a silver–alumina–graphene oxide nanocomposite ($GO/Ag/Al_2O_3$), against clinical isolates of *Aspergillus* species. The nanoparticles were synthesized using a chemical method and characterized by UV–Vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), field-emission scanning electron microscopy (FESEM), dynamic light scattering (DLS), and energy-dispersive X-ray spectroscopy (EDX). A total of 25 clinical isolates representing *A. flavus, A. terreus, A. niger, A. tubingensis*, and *A. fumigatus* were included in the study. The minimum inhibitory concentrations (MICs) of the nanoparticles were determined using the CLSI M38-A2 broth microdilution method. The cytotoxic effects of the nanoparticles were evaluated using the MTT assay. The GO nanoparticles did not exhibit significant antifungal activity against any of the tested isolates, with MIC values ≥500 µg/mL and no complete growth inhibition observed. In contrast, the Ag/Al_2O_3 demonstrated the strongest antifungal activity, with MIC values ranging from 62.5 to ≥500 µg/mL (MIC_{50} = 312.5 µg/mL, MIC_{90} = 500 µg/mL, and geometric mean MIC = 217.63 µg/mL). The $GO/Ag/Al_2O_3$ also exhibited considerable antifungal activity, with MIC values ranging from 125 to ≥500 µg/mL (MIC_{50} = 250 µg/mL, MIC_{90} = 500 µg/mL, and geometric mean MIC = 280.61 µg/mL). Among the tested nanomaterials, the Ag/Al_2O_3 exhibited the strongest antifungal activity against clinical isolates of *Aspergillus* species. The incorporation of GO into the Ag/Al_2O_3 nanocomposite did not result in a significant enhancement of antifungal efficacy. These findings highlight the potential of Ag/Al_2O_3 nanocomposites as promising antifungal agents for the management of aspergillosis.

Keywords: *Aspergillus*, graphene oxide, silver–alumina nanocomposite, nanoparticles, antifungal activity

A report of a rare yeast from a tertiary care hospital: *Petasospora fabianii*

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Abstract

Invasive fungal infections are major global health concerns, especially in hospitalized and immunosuppressed patients. Although *Candida albicans* is predominant, rare species like the opportunistic ascomycetous yeast *Petasospora fabianii* (*Cyberlindnera fabianii)* are increasingly isolated. We aim to evaluate the clinical data and antifungal susceptibility of *P. fabianii* isolates at Uşak Training and Research Hospital. All *P. fabianii* strains isolated between September 2025 and June 2026 were retrospectively analyzed. Duplicate strains were excluded. Identification was performed via MALDI-TOF MS (Vitek MS Prime), and minimum inhibitory concentrations (MICs) were determined using Sensititre YO10 microdilution. Data were analyzed using SPSS v27.0. Out of 43 isolates (42 urine, 1 bronchoalveolar lavage), 28 (65.1%) patients were male and 15 (34.9%) were female, with a mean age of years. All patients had an intensive care unit history. The overall mortality rate was 65.1% (28/43). Mortality was 41.7% in the colonization group compared to 74.2% in the active infection group (p value:0.074). No therapeutic agent (amphotericin B, echinocandin, or fluconazole) showed a statistical superiority regarding survival (p value>0.05). Antifungal susceptibility testing was performed on 39 isolates, excluding 4 that failed to regrow. Our study shows that *P. fabianii* is a critical opportunistic pathogen in elderly, ICU-admitted patients, dramatically increasing mortality during active infections. Among the studies reporting *P. fabianii* in our country, Sariguzel et al. documented 29 isolates, whereas Turan et al. reported 24 isolates. As one of the largest series from Turkey, this study underscores the necessity of advanced identification tools like MALDI-TOF MS and laboratory-based surveillance to guide rational antifungal therapy.

Keywords: *Petasospora fabianii*, minimum inhibitory concentration, invasive fungal infection

Methods Used for the Identification of *Candida* spp. and Changing Taxonomy

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Abstract

Within the field of mycology, it is known that while there are millions of fungal species-tens of thousands of which cause diseases in living organisms-only about 300 species are responsible for infections and allergic reactions in humans. Although the number of species within the fungal kingdom that exhibit pathogenicity in humans is small, fungal infections pose a significant threat to human health. Approximately 2 million people worldwide lose their lives annually due to fungal infections, particularly among immunosuppressed patient groups. When fungal infections are considered, invasive candidiasis-caused specifically by *Candida* spp. ranks at the forefront of infection-related deaths. Notable species responsible for these infections include *Candida albicans*, *Nakaseomyces glabratus* (*C. glabrata*), L*odderomyces parapsilosis* (*C. parapsilosis*), *Issatchenkia orientalis* (*C. krusei*), *Candidozyma auris* (*C. auris*) and *C. tropicalis*. Since these species pose a significant risk to human health, the World Health Organization has classified them as pathogens representing a critical threat. Consequently, the accurate identification of these pathogens is crucial for both epidemiological purposes and the initiation of the most appropriate antifungal therapy for patients. Although various diagnostic methods exist, the traditional culture method remains the gold standard for diagnosing invasive *Candida* spp. infections. Clinical samples-such as blood, urine and urogenital or skin swabs are collected under sterile conditions and inoculated onto Sabouraud Dextrose Agar and CHROMagar *Candida* media; following incubation for 1-3 days at 25°C or 37°C (depending on the sample type), colonies exhibiting significant growth undergo microscopic and phenotypic examination. As part of the microscopic analysis, the germ tube test specifically used to distinguish *C. albicans* from other *Candida* species is frequently employed in routine practice; in this test fungal colonies suspended in human blood serum are incubated at 37°C for 3 hours, and microscopic observation of a germ tube structure indicates the species is *C. albicans*, whereas its absence indicates another *Candida* species. Subsequently, species identification of the pathogen is performed using automated identification systems such as VITEK. Additionally, the MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization -Time of Flight) mass spectrometry method is widely used for definitive species identification. Molecular testing methods offer the fastest and most reliable results among the available techniques. These molecular methods are of great importance, as they enable early diagnosis in cases of severe infection and in situations where conventional culture methods prove inadequate. In this context, the Panfungal PCR (Polymerase Chain Reaction) test which enables the rapid and reliable detection of a wide variety of fungal species in a single test period by targeting the DNA or RNA of a broad fungal group and the *Candida* PCR test which screens for *Candida* spp. DNA in samples obtained from infected tissues or body fluids are important tests currently in use.

Keywords: *Candida*, fungal Infections, identification methods, fungal taxonomy

Cryptococcus Species Isolated from the External Auditory Canal in Dogs and Cats: Commensal Flora or Opportunistic Pathogens?

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Abstract

Naganishia species are microorganisms formerly classified within the genus *Cryptococcus* and long considered environmental saprophytic yeasts. However, the reliance of routine diagnostic systems on outdated *Cryptococcus* taxonomy, along with their limited discriminatory power at the species level, hinders the elucidation of the clinical significance of these yeasts. This study aimed to compare the species-level diagnostic performance of methods used to identify *Cryptococcus*-like yeasts isolated from the external ear canals of cats and dogs, and to evaluate the clinical distribution of the identified species. A total of 14 yeast isolates, which were initially identified as *Cryptococcus* spp. by routine methods, were evaluated. These isolates were obtained from the external ear canals of cats and dogs with otitis externa (n = 7) and from clinically healthy animals (n = 7). The methods employed included API ID 32C, VITEK 2 Compact, MALDI-TOF MS and ITS and D1–D2 LSU sequence analyses. ITS sequence analysis was accepted as the reference method for species-level identification, and phylogenetic analyses were performed using the Maximum Likelihood algorithm implemented in MEGA12. The clinical distribution, antifungal minimum inhibitory concentrations (according to CLSI M27-A3), and epidemiological characteristics were assessed. Statistical analyses were conducted using SPSS 23.0 ($p < 0.05$). ITS sequence analysis differentiated the isolates into four *Naganishia* species (*N. adeliensis* 28.6%, *N. albida* 14.3%, *N. albidosimilis* 28.6%, and *N. uzbekistanensis* 28.6%) and formed species-specific monophyletic clades. The D1–D2 LSU analysis was successful at the genus and complex levels, but showed lower discriminatory power than ITS at the species level. The highest level of agreement at the species level was achieved with MALDI-TOF MS (57.1%; κ=0.455), whereas API ID 32C (14.3%; κ=0.000) and VITEK 2 Compact (14.3%; κ=0.056) showed lower agreement. *N. adeliensis* and *N. albida* were isolated only from animals with otitis. *N. uzbekistanensis* was isolated only from healthy animals (100%). *N. albidosimilis* was predominantly isolated from healthy animals (75%). Antifungal

susceptibility testing revealed variable MIC distributions for four antifungal agents. The greatest variability was observed for itraconazole, while amphotericin B, fluconazole and flucytosine showed narrower MIC ranges. ITS sequence analysis was considered the most reliable method for the accurate identification of *Naganishia* species, while MALDI-TOF MS was identified as the most effective alternative for routine laboratory use. The observed differences in the clinical distribution of the species indicate that *Cryptococcus/Naganishia* isolates from the external ear canal should not be regarded as a uniform commensal flora. These findings highlight the critical importance of species-level identification for clinical interpretation and underscore the need for further investigation of species-specific pathogenicity and host–microorganism interactions.

Keywords: *Naganishia*, API ID 32C, VITEK 2 Compact, MALDI-TOF MS, ITS and D1–D2 LSU sequence analysis

Development and Characterization of *Usnea florida* Extract-Loaded Sodium Alginate Hydrogel Film with Antimicrobial and Antioxidant Activities

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Abstract

Lichens have long been used in traditional medicine for the treatment of skin infections, wounds, and superficial tissue damage owing to their wound-healing, antimicrobial, and hemostatic properties. This traditional knowledge has inspired scientific investigations into their biological potential. In this study, a sodium alginate-based hydrogel film containing extracts of the epiphytic macrolichen *Usnea florida* (L.) F.H. Wigg. was developed as a natural wound dressing candidate. Based on preliminary screening, the ethanol extract, which exhibited superior biological activity, was selected for hydrogel formulation. Antioxidant activities of the lichen extract and extract-loaded hydrogel were evaluated using DPPH, FRAP, and CUPRAC assays, while total phenolic content was determined by the Folin–Ciocalteu method. Antimicrobial activity of both the extracts and hydrogel formulation was assessed against wound-associated pathogens using agar well diffusion and broth microdilution assays. The prepared hydrogel was further characterized by evaluating its in vitro release profile together with its antimicrobial and antioxidant performances. The *Usnea florida* ethanol extract-loaded hydrogel demonstrated enhanced biological activity, particularly against *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus mutans*, and *Enterococcus faecalis*, compared with the free extract. Furthermore, the hydrogel maintained considerable antioxidant capacity, supporting its potential to reduce oxidative stress during wound healing. Overall, the developed *Usnea florida*-loaded sodium alginate hydrogel represents a promising natural biomaterial for wound management due to its combined antimicrobial and antioxidant properties. Nevertheless, further in vitro and in vivo studies are required to confirm its efficacy, safety, and clinical applicability.

This study was supported by TÜSEB (Project no: 48366).

Keywords: Lichens, *Usnea florida*, natural biomaterials, hydrogel film, wound healing

Species Distribution and Antifungal Susceptibility Profiles in Clinical *Candida* Isolates: A Single-Center Experience from Edirne

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Abstract

Candida species are among the leading opportunistic agents of invasive fungal infections, particularly in intensive care unit (ICU) patients. The increasing frequency of non-albicans *Candida* species and shifts in antifungal susceptibility profiles have heightened the clinical importance of accurate species-level identification and antifungal susceptibility testing. This study retrospectively evaluated the distribution and antifungal susceptibility profiles of *Candida* species isolated from clinical specimens at Edirne Sultan 1. Murat State Hospital. In this retrospective study, *Candida* strains isolated from clinical specimens submitted to the Medical Microbiology Laboratory were analyzed and repeat isolates from the same patient were excluded. Species-level identification was performed using the BD Phoenix™ M50 system, and antifungal susceptibility testing with the Sensititre™ YeastOne™ system. Minimum inhibitory concentration (MIC) values were interpreted according to EUCAST clinical breakpoints; for antifungals lacking EUCAST breakpoints, CLSI breakpoints were used. Data were analyzed using descriptive statistics. Between April and July 2026, 59 yeast isolates were included. Of the patients, 33 were female (mean age: 73.90 years) and 26 were male (mean age: 70.70 years); 80% (n=47) of isolates originated from ICUs. The most frequent source was urine (n=42), followed by blood (n=12), tracheal aspirate (n=3), tissue (n=1), wound (n=1), and abscess (n=1). The predominant species were *Candida tropicalis* (n=17), *Candida albicans* (n=16), and *Candida auris (Candidozyma auris)* (n=11), followed by *Candida glabrata (Nakaseomyces glabratus)* (n=9), *Candida parapsilosis* (n=2), *Candida krusei* (Issatchenkia orientalis) (n=1), and *Candida kefyr* (*Kluyveromyces marxianus)* (n=1); one isolate was *Saprochaete capitata*, and one was unidentified. Based on the evaluation of species and antifungals with defined clinical breakpoints in guidelines, *Candida albicans* isolates exhibited 100% susceptibility to micafungin, caspofungin, and amphotericin B, while susceptibility rates were calculated as 81% for posaconazole, voriconazole, itraconazole, and fluconazole, and 50% for anidulafungin. For *non-albicans Candida* species, the highest susceptibility rates were observed for micafungin (82%), caspofungin (68%), amphotericin B (66%), and anidulafungin (56%), whereas markedly lower susceptibility rates were determined for voriconazole (16%), itraconazole (14%), fluconazole (14%), and posaconazole (11%). Our study revealed that *non-albicans Candida* species were predominant among intensive care unit-derived isolates and that antifungal susceptibility profiles varied significantly across species. These findings underscore the critical importance of accurate species identification and antifungal susceptibility testing in the management of fungal infections, suggesting that regional

surveillance data will contribute substantially to empirical treatment strategies and appropriate antifungal selection.

Keywords: Antifungal susceptibility, *Candida* species

In Vitro Antifungal Activity of *Aronia melanocarpa* Extract Alone and in Combination with Fluconazole against *Candida albicans*

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Abstract

Candida albicans is one of the leading opportunistic fungal pathogens, and the increasing occurrence of reduced susceptibility to fluconazole necessitates the investigation of alternative therapeutic approaches. *Aronia melanocarpa* has been widely investigated because of its potential biological and antimicrobial properties. The present study aimed to evaluate the in vitro antifungal activity of *Aronia melanocarpa* extract alone and in combination with fluconazole against *C. albicans* isolates. Commercial powdered *Aronia melanocarpa* extract (Nilkim Chemical, Turkey) was dissolved in sterile distilled water before use. Antifungal susceptibility testing was performed by the broth microdilution method according to CLSI M27 guidelines using one reference strain (*C. albicans* ATCC 10231) and five clinical *C. albicans* isolates. The minimum inhibitory concentrations (MICs) of fluconazole and aronia extract were determined individually. Drug interactions were evaluated using the checkerboard microdilution method, and fractional inhibitory concentration index (FICI) values were calculated to determine the interaction type. Fluconazole MIC values ranged from 0.125 to 16 µg/mL, whereas aronia extract MIC values ranged from 50 to 400 µg/mL. Combination testing resulted in 2–8-fold reductions in fluconazole MICs relative to fluconazole alone. Checkerboard analysis demonstrated additive interactions in the *C. albicans* ATCC 10231 reference strain and four of the five clinical isolates, whereas one clinical isolate exhibited a synergistic interaction (FICI = 0.5). No antagonistic interactions were observed. *Aronia melanocarpa* extract exhibited favorable in vitro interactions with fluconazole against *C. albicans*, demonstrating predominantly additive and occasionally synergistic effects without evidence of antagonism. These findings suggest that aronia extract may serve as a promising natural adjunct to fluconazole therapy. Further studies including larger numbers of clinical isolates and mechanistic investigations are needed to confirm these findings.

Keywords: *Aronia melanocarpa, Candida albicans*, fluconazole, antifungal activity, checkerboard method

Species Distribution, Species Characterization, and Antifungal Susceptibility of Clinical Dermatophyte Isolates

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Abstract

The global epidemiology of dermatophytosis is rapidly changing due to the emergence of antifungal-resistant dermatophytes. Epidemiological surveillance has highlighted the need for accurate species-level identification and interaction between clinical microbiology laboratories and clinicians for treatment decisions and early detection of resistant strains. This study evaluated the epidemiological characteristics, anatomical distribution, and antifungal susceptibility profiles of dermatophyte infections encountered in routine clinical practice, and also aimed to investigate the complementary roles of MALDI-TOF MS and traditional mycological methods in routine clinical practice. In the Medical Microbiology and Mycology Laboratory of Istanbul University-Cerrahpaşa Faculty of Medicine Hospital, 126 dermatophyte isolates obtained from clinical samples of superficial mycoses between 2017-2018 were analyzed using conventional and MALDI-TOF methods. Patients with recurrent infections underwent antifungal susceptibility testing. Tinea corporis was the predominant clinical presentation, accounting for 41.3% of cases, followed by tinea unguium (30.2%) and tinea pedis (11.9%). MALDI-TOF MS provided reliable species-level identification for 71 (56.3%) of 126 isolates, while the remaining 55 isolates (43.7%) were characterized using conventional mycological methods. Conventional methods identified 39 isolates as *Trichophyton mentagrophytes*, four as *Microsporum canis*, three as *Trichophyton rubrum*, two as *Trichophyton tonsurans*, one as *Microsporum audouinii,* and one as *Trichophyton violaceum*, while five isolates were reported as dermatophytes spp. Overall, *T. rubrum* was the most frequently isolated species (48.4%), followed by *T. mentagrophytes* (31.7%). Antifungal susceptibility testing showed variable susceptibility profiles among isolates from persistent or recurrent cases. MALDI-TOF MS and conventional phenotypic methods should be considered complementary approaches for dermatophyte identification. The significant contribution of conventional methods to species identification underscores their continued importance in routine clinical mycology laboratories, particularly in situations where commercial MALDI-TOF MS databases are incomplete. Integrating identification and susceptibility testing improves the management and monitoring of resistant dermatophytosis.

Keywords: Dermatophytes, conventional methods, identification, MALDI-TOF MS, antifungal susceptibility

Chaxines from the edible mushroom *Agrocybe chaxingu* engage the RORγt inverse-agonist pocket: an in silico docking and ADMET study

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Abstract

The retinoic acid receptor-related orphan receptor gamma t (RORγt) is the master transcription factor of Th17 differentiation and, through the Th17/IL-17 axis, a validated small-molecule target in autoimmune and inflammatory diseases including psoriasis, psoriatic arthritis, ankylosing spondylitis, inflammatory bowel disease and rheumatoid arthritis; its ligand-binding domain (LBD) accommodates sterols, and natural products such as diosgenin act as RORγt inverse agonists. Chaxines are ergosterol-derived degraded sterols from the edible mushroom *Agrocybe chaxingu* with documented osteoclast-formation–suppressing activity, and because the RORγt-driven axis also promotes osteoclastogenesis and inflammatory bone loss, RORγt is a plausible but untested molecular target for these metabolites. Chaxines B–E were evaluated as candidate RORγt ligands by structure-based docking and consensus ADMET profiling. Verified structures were docked into the RORγt LBD inverse-agonist pocket (PDB 9N9L, 1.64 Å) using AutoDock Vina with triplicate seeds, benchmarked against the co-crystallized clinical inverse agonist A1BW2 and the fungal sterol ergosterol; the protocol was validated by redocking A1BW2 (RMSD 1.49 Å), and ADMET was assessed with SwissADME, ADMETlab 3.0 and ProTox 3.0. All chaxines occupied the same orthosteric pocket as A1BW2, contacting key residues His479, Trp317 and Phe377/378. Their binding affinities (−9.7 to −10.3 kcal/mol) were sterol-like, below the reference A1BW2 (−11.3) and comparable to ergosterol (−10.3), with chaxine B ranking highest. The chaxines showed favourable predicted acute toxicity (ProTox class 5; LD50 4216 to 6400 mg/kg), markedly safer than ergosterol (class 2), together with good intestinal absorption and low mutagenicity, while high lipophilicity remained the main liability. Overall, these mushroom-derived metabolites engage the validated RORγt inverse-agonist pocket with clinical-ligand-like contacts and a favourable safety profile, supporting them as starting scaffolds for Th17/IL-17-axis modulation; experimental and molecular-dynamics validation are warranted.

Keywords: RORγt, molecular docking, *Agrocybe chaxingu*, chaxines, Th17/IL-17 axis

Rodlet- and DHN-Melanin-Associated Gene Expression Profiles and Cytotoxicity in Clinical *Aspergillus fumigatus* Strains

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Abstract

Aspergillus fumigatus is one of the leading causative agents of invasive aspergillosis and represents an important opportunistic fungal pathogen associated with high mortality in immunocompromised patients. The rodlet layer and DHN-melanin on the conidial surface play key roles in immune evasion, environmental stress resistance, and fungal virulence. This study aimed to investigate the expression of rodlet-associated (rodA, rodB, rodC, rodD, rodE, and rodF) and DHN-melanin biosynthesis-related (pksP/alb1, abr1, abr2, arp1, and arp2) genes in clinical *A. fumigatus* strains and to evaluate these findings together with conidial surface morphology and cytotoxicity. Twenty-nine clinical *A. fumigatus* strains obtained from immunocompromised patients with probable invasive aspergillosis according to Infectious Diseases Society of America (IDSA) criteria, together with one reference strain, were included. Species identification was confirmed by ITS PCR and sequencing. Relative gene expression levels were determined by RT-qPCR using the 2^-ΔΔCt method with 18S rRNA as the reference gene. Conidial surface morphology was examined by scanning electron microscopy (SEM), and cytotoxicity was evaluated in the WI-38 cell line using the MTT assay. Heterogeneous expression profiles were observed for rodA, rodB, rodE, and rodF, whereas rodC showed no amplification and rodD expression was detected only in a limited number of strains. The highest rodA expression was observed in strain 19. Among DHN-melanin biosynthesis genes, the highest pksP (alb1) expression was detected in strain 28, whereas abr1, abr2, and arp2 exhibited the highest expression in strain 29. arp1 amplification was detected in only one strain. SEM analyses revealed more organized conidial surfaces in strains with high rodlet gene expression. Cytotoxicity assays demonstrated that gene expression levels were not consistently correlated with cytotoxic effects. Clinical *A. fumigatus* strains exhibited heterogeneous rodlet- and DHN-melanin-associated gene expression profiles. Integrating molecular, morphological, and functional analyses provides a comprehensive understanding of fungal virulence and may contribute to future studies investigating pathogenic mechanisms and potential antifungal targets.

Keywords: *Aspergillus fumigatus*, rodlet layer, DHN-melanin, RT-qPCR, SEM, cytotoxicity

First Detection of *Candida auris* (*Candidozyma auris*) Candidemia at Kırklareli Training and Research Hospital: A Three-Case Experience

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Abstract

Candida auris (Candidozyma auris) has emerged as an important nosocomial fungal pathogen because of its multidrug resistance, propensity for healthcare-associated transmission, and high mortality. We report the first three consecutive cases of *C. auris* candidemia identified at Kırklareli Training and Research Hospital and describe their clinical, microbiological, and therapeutic characteristics. Blood cultures were processed using an automated blood culture system (Render BC128). Positive cultures were identified as *C. auris* using the bioMérieux VITEK 2 Compact system, and antifungal susceptibility testing was performed. All isolates were resistant to fluconazole and susceptible to echinocandins and amphotericin B. The first patient was a 45-year-old man with prolonged intensive care unit (ICU) hospitalization following intraventricular hemorrhage. Despite fever, inflammatory markers were only mildly elevated (WBC: 4,470/mm³; CRP: 8 mg/L; procalcitonin: 3.12 ng/mL). Appropriate antifungal therapy resulted in microbiological clearance and clinical recovery. The second patient was a 71-year-old man with advanced lung and laryngeal carcinoma. Blood cultures obtained because of persistent inflammatory response (WBC: 12,850/mm³; CRP: 181 mg/L) yielded *C. auris,* while a concurrent tracheal aspirate culture grew multidrug-resistant *Pseudomonas luteola.* Despite antifungal therapy, the patient died because of advanced malignancy. The third patient was a 50-year-old man admitted to the ICU with acute coronary syndrome. Blood cultures yielded *C. auris* following clinical deterioration (WBC: 14,940/mm³; CRP: 53 mg/L). Although the initial episode resolved after antifungal therapy, recurrent candidemia developed approximately one month later, and the patient died despite repeated treatment. All three patients had prolonged ICU stay, invasive procedures, and previous exposure to broad-spectrum antimicrobials. The temporal occurrence of these cases following the transfer of a patient with *C. auris* infection from another hospital highlights the need for heightened awareness of possible healthcare-associated transmission. Early species-level identification, antifungal susceptibility testing, prompt initiation of appropriate therapy, and strict infection prevention and control measures are essential for the management of *C. auris* candidemia.

Keywords: *Candida auris*, candidemia, intensive care unit, infection prevention and control

Sustainable Use of Mushrooms in Pharmaceutical Technology

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Abstract

Mushrooms have long been recognized as valuable natural resources, serving not only as nutritious foods but also as therapeutic agents in traditional medicine. Their diverse repertoire of bioactive compounds, including phenolics, polysaccharides, and triterpenes, has attracted growing scientific interest for pharmaceutical applications. Recent studies highlight their anticancer, immunomodulatory, and hemostatic properties, underscoring the potential of innovative formulations derived from mushroom extracts. Within this framework, the integration of green chemistry principles and sustainability offers an environmentally responsible pathway for developing mushroom-based pharmaceutical products. In our research, several edible and medicinal species were investigated to elucidate their biological potential. Extracts of *Cantharellus cibarius*, *Craterellus cornucopioides*, *Ramaria formosa*, and *Hydnum repandum* were analyzed by liquid chromatography for phenolic content and tested for antimicrobial and anticancer activities. Among these, *H. repandum* demonstrated remarkable antioxidant and antimicrobial effects, attributable to its high chlorogenic and gallic acid levels. Furthermore, it exhibited selective cytotoxicity against hepatocellular carcinoma cells, inducing apoptosis through upregulation of Bax, p53, and caspase-3, while downregulating cyclin D1 expression. These findings emphasize the potential of mushroom phenolics as sustainable anticancer agents. In parallel, a gel formulation derived from *Fomes fomentarius* (Amadou mushroom) was developed to assess its hemostatic efficacy. In rat bleeding models, the gel significantly reduced hemostasis and coagulation times by up to 70%, demonstrating promise as a topical agent for bleeding control. Antimicrobial assays further confirmed inhibitory activity against *Klebsiella pneumoniae* and several *Candida* species. Beyond anticancer and hemostatic perspectives, mushrooms are increasingly recognized as nutraceuticals with immunomodulatory properties. Polysaccharides such as β-glucans enhance immune function by stimulating macrophages and cytokine production, offering synergistic benefits in cancer immunotherapy. Collectively, our work demonstrates that mushroom extracts represent a multifaceted and sustainable platform for innovative pharmaceutical formulations, bridging traditional knowledge with modern biomedical applications through the lens of green chemistry.

Keywords: Mushroom extracts, anticancer activity, immunomodulation, hemostatic formulations, green chemistry, sustainable pharmaceutical technology

Evaluation of the Distribution of Environmental Saprophytic Fungi and Yeasts Isolated from Clinical Specimens

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Abstract

This study aimed to evaluate the distribution of rare and predominantly saprophytic fungi and yeasts isolated from clinical specimens. Saprophytic fungi isolated in our laboratory were included in the study between January 1, 2024, and August 31, 2026. Yeasts were evaluated between September 2025 and August 31, 2026 with the MALDI-TOF MS method. Conventional methods were used for the genus-level identification of saprophytic fungi, whereas species-level identification was performed using DNA sequence analysis. *Lomentospora prolificans*, *Moniliella suaveolens* var. *nigra*, and *Maudiozyma turicensis* (formerly *Kazachstania turicensis*) were isolated from sputum; *Trichoderma citrinoviride* and *Pichia kluyveri* from endotracheal aspirates; *Aspergillus nidulans* from a corneal swab; *Acremonium sclerotigenum* and *Scopulariopsis brevicaulis* from nail specimens; and *Kloeckera apiculata* from a blood culture. The literature review revealed that there were no reports at the species level of *L. prolificans* and *M. suaveolens var. nigra* from sputum samples in Türkiye, nor of *A. sclerotigenum* and *S. brevicaulis* from nail samples in Türkiye. *K. apiculata*, which we isolated from a blood culture, is the first isolate reported from a clinical specimen in Türkiye. *A. nidulans*, which was previously reported from a case of endophthalmitis in Türkiye, was isolated from a corneal swab of a patient diagnosed with keratitis in our study. *T. citrinoviride,* which has been reported to be isolated from BAL samples, was obtained from an endotracheal aspirate in our study; to the best of our knowledge, this is the first report in this clinical specimen. *M. turicensis, which we isolated from sputum,* and *P. kluyveri,* which we obtained from endotracheal aspirate, are the first reported isolates of these species from these types of clinical specimens. In this context, these findings will contribute to the literature in Türkiye and worldwide.

Keywords: *Trichoderma citrinoviride*, *Maudiozyma turicensis*/*Kazachstania turicensis*, *Pichia kluyveri*, *Moniliella suaveolens* var. *nigra*, Türkiye

Fungal Systematics, Taxonomy and Nomenclature

Two New Records of Powdery Mildew Fungus for Türkiye

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Abstract

Erysiphe symphoricarpi (Howe) U. Braun & S. Takam., on *Symphoricarpos albus* (L.) S.F.Blake (Caprifoliaceae) and *Phyllactinia parietariae* U. Braun & Khodap on *Parietaria judaica* L. (Urticaceae) are reported for the first time from Elazığ province in Türkiye. The morphological and microscopical features of this fungi is described with figures.

This study was supported by TÜBİTAK (Project No: 223Z275)

Keywords: Parasitic microfungi, taxonomy, Türkiye, biodiversity, Elazığ

The new record from Hakkâri (Türkiye) *Pachyella hydrophila* (Sacc.) Pfister

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Abstract

This study reports the first record of the rare discomycete (cup fungus) species *Pachyella hydrophila* (Sacc.) Pfister from Türkiye. Macromycete specimens were collected in 2025 from a wooded area adjacent to a KYK student dormitory in Mezran neighborhood, Hakkâri Province, at an elevation of approximately 1,770 m. The species was originally described by C. H. Peck in 1880 from the Adirondack Mountains of New York State. Prior to this study, only two species belonging to the genus Pachyella had been recorded from Türkiye; consequently, the genus is now represented by three species in the country. The present study provides a detailed description of *Pachyella hydrophila* (Sacc.) Pfister, together with macro- and micromorphological photographs of the species.

Keywords: *Pachyella hydrophila*, Discomycetes, cup fungus, Ascomycota, new record, Hakkâri, Türkiye, macrofungi

Systematics and Ecology of the Genus *Geastrum*: A Comprehensive Review

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Abstract

The genus *Geastrum* has been subject to numerous studies since the 20th century. However, due to geographical biases, general knowledge gaps, and limited access to some key resources on the genus, a comprehensive synthesis is required. The inter- and intrageneric phylogenies of *Geastrum* have changed since the genus was first proposed in 1794, with the addition of sections and subsections in recent years. Furthermore, many new species are described every year. However, these species are usually classified based on morphological characters, and species boundaries are often uncertain, leading to an overestimation of diversity and the proliferation of taxonomic synonyms. Information on the ecology of *Geastrum* remains limited and is largely restricted to habitat records and reports of enzymatic activity. Consequently, important questions regarding the ecology of the genus remain unresolved, including the nature of its interactions with plants and the trophic strategies of some species. This review synthesizes information from 79 publications, including peer-reviewed papers, taxonomic monographs, and selected academic theses, with the aim of evaluating current knowledge of the taxonomy, ecology, and distribution of *Geastrum* and identifying priorities for future research. The most commonly used taxonomic characters are associated with the genus' distinctive star-like basidiomata and include variation in the shape, size, texture, and structure of both the endoperidium and exoperidium. Basidiospores and hyphae have more recently been considered key characters for species delimitation with the advent of Scanning Electron Microscopy. Derived from this technology, calcium oxalate crystals have been observed in rhizomorphs and have been proposed as taxonomically informative characters due to their apparent stability across environmental conditions. In addition, the verrucose ornamentation of spores may also be used as a diagnostic character. At a finer scale, several DNA regions are commonly employed for both species identification and population genetics, with an increasing number of studies emphasizing the importance of integrative approaches that combine multiple loci simultaneously. Overall, the available evidence indicates that many *Geastrum* species described solely on morphological characters should be re-evaluated using integrative approaches. This is necessary to create a more stable taxonomy and may reveal previously unrecognized diversity in several regions of the world. Furthermore, additional research on trophic strategies and other ecological aspects of the genus is needed to address persistent gaps in our understanding of *Geastrum* biology. This is necessary to contribute to a more stable taxonomy and may reveal previously unrecognised diversity in several regions of the world.

Keywords: *Geastrum*, taxonomy, ecology, systematics, morphology

Pholiotina mediterranea (Bolbitiaceae, Agaricales): First Record for the Mycobiota of Türkiye Confirmed by Morphological and ITS Sequence Analyses

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Abstract

Pholiotina mediterranea Siquier & Salom is a recently described and rarely reported Mediterranean agaricoid fungus. Owing to its small basidiomata and morphological similarity to allied taxa, accurate species identification requires the integration of morphological and molecular data. During macrofungal biodiversity surveys conducted in northwestern Türkiye, specimens corresponding to *P. mediterranea* were collected and examined using an integrative taxonomic approach. Basidiomata were collected on 22 January 2025 from moss-covered ground around the Rectorate area of Terzioğlu Campus, Çanakkale Onsekiz Mart University (Çanakkale, Türkiye). Detailed macro- and micromorphological examinations were performed using standard mycological methods. Genomic DNA was extracted from dried material, and the nuclear ribosomal ITS region was amplified and sequenced. Species identification was confirmed through comparative sequence analyses together with morphological evaluation. The ITS sequence obtained from the Turkish collection was 641 bp in length. BLASTn analysis revealed the closest matches with previously published *P. mediterranea* sequences, showing up to 99.69% sequence identity, 100% query coverage, and an E-value of 0.0. These molecular data, together with diagnostic morphological characters, confirmed the identification of the specimen as *P. mediterranea*. Based on the available literature and national fungal checklists, this taxon is reported here as a new record for the mycobiota of Türkiye. This study contributes to the fungal biodiversity of Türkiye by documenting a previously unreported member of Bolbitiaceae using an integrative taxonomic framework. The findings emphasize the importance of combining detailed morphological investigations with ITS-based molecular analyses for the accurate recognition of morphologically similar agaricoid fungi and provide new insights into the distribution of Pholiotina mediterranea in the Mediterranean Basin.

Keywords: Agaricoid fungus, biodiversity, Bolbitiaceae, integrative taxonomy, ITS, new record, Pholiotina, Türkiye

On the host range and diversity of the *Hyaloperonospora brassicae* complex

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Abstract

Downy mildews are a group of more than 700 species of obligate parasites that infects both crops and wild plants. The third largest group among them is the brassicolous downy mildews with more than 100 known species. The Hyaloperonospora brassicae complex includes species that cause downy mildews on various Brassicaceae plants, such as cabbage, rapeseed, mustard, and rocket. Although some species in the Hyaloperonospora brassicae complex have been reported in previous studies and some information has been obtained about the host spectrum of this species complex, no comprehensive study on the Hyaloperonospora brassicae complex has been conducted. Thus, the question of whether this complex may include more pathogen species and hosts than currently known formed the basis of this study. For addressing this question, more than 200 specimens obtained from 1978 to 2025, originating from Sweden, Germany, Greece, Spain, Italy, and Türkiye were used. Multilocus phylogenetic analyses revealed unknown species within this complex. The morphological characterization of these new species were completed. In addition, new results regarding their host ranges were obtained. The finding that a host can be infected by more than one species within the Hyaloperonospora brassicae complex, and the identification of a species with a wide host range, has immediate implications for understanding the epidemiology of downy mildew of Brassicaceae crops.

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Keywords: *Hyaloperonospora*, host, new species

Artificial Intelligence, Image Analyses and Digital Mycology

Classification of Macrofungal Genera from Basidiospore Micrographs Using CLAHE-Enhanced DenseNet121

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Abstract

This study presents a CLAHE-DenseNet121 approach that integrates image enhancement and deep learning for the automatic classification of basidiospore micrographs belonging to the genera *Agaricus, Amanita, Hebeloma, Inocybe* and *Russula*. The dataset consists of 1,603 microscopic images obtained from 50 biological specimens representing five genera. In the first stage, ResNet18, InceptionV3, MobileNetV2, EfficientNetB0, DenseNet121 and VGG16 were compared using out-of-fold predictions, and the highest baseline performance was achieved by DenseNet121 (ACC = 0.8939; F1-Macro = 0.8941; MCC = 0.8675). In the second stage, RGB images were converted into the LAB color space, CLAHE was applied only to the luminance-carrying L channel, and the enhanced images were fed into DenseNet121. The proposed approach was evaluated using 3-fold stratified cross-validation; the mean accuracy, F1-Macro, MCC, ROC-AUC and PR-AUC were 0.9258 +/- 0.0066, 0.9262 +/- 0.0061, 0.9072 +/- 0.0081, 0.9916 +/- 0.0014 and 0.9735 +/- 0.0022, respectively. The best fold reached an accuracy of 0.9326. The findings indicate that controlled local contrast enhancement helps DenseNet121 learn discriminative micromorphological patterns such as spore boundaries, wall structure, pigmentation and surface ornamentation more effectively.

Keywords: Macrofungi, basidiospore, microscopic image, CLAHE, DenseNet121, deep learning, image classification

Hybrid CNN-Transformer Architecture for Automatic Classification of Mushroom Species Using Deep Learning

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Abstract

This study addresses the automatic classification of macrofungal species using deep learning-based methods. Because conventional mushroom identification is expertise-dependent and time-consuming, image-based automatic recognition systems offer an important alternative for biodiversity research and food safety. Within this scope, convolutional neural network (CNN)-based architectures such as ResNet50, DenseNet121 and VGG16 were comparatively evaluated together with modern deep learning models such as ConvNeXtV2, Swin Transformer and Vision Transformer (ViT). In addition, a novel hybrid architecture that combines CNN-based local feature extraction with Transformer-based global context modelling is proposed. In the proposed model, image features are extracted through a ConvNeXtV2 backbone; global relationships are then modelled using a Transformer encoder and cross-attention mechanisms, and a unified representation is generated through adaptive fusion. Experimental studies were conducted on an image dataset consisting of 11 mushroom classes. The results show that the proposed hybrid model achieved higher performance than all comparison methods, with 96.58% accuracy and a Macro F1 score of 0.9639. The findings indicate that the joint use of CNN- and Transformer-based approaches can provide stronger representation learning in biological image classification problems. The proposed approach has potential not only for automatic identification of mushroom species but also for different computer vision applications such as plant disease diagnosis, biological species classification and medical image analysis.

Keywords: Macrofungus classification, deep learning models, CNN-Transformer hybrid architecture, ConvNeXtV2, Vision Transformer (ViT), image classification

Slime Moulds as a Universal Model for Minimal Cognition, Decentralized Computing, and Bio-Industrial Sustainability

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Abstract

Slime moulds (*Myxomycetes*), particularly the acellular plasmodial species *Physarum polycephalum*, have evolved from biological curiosities into valuable models for unconventional computing, sustainable biotechnology, and biomedical research. The primary objective of this narrative review is to uncover the lesser-known physiological, ecological, and adaptive mechanisms of true slime moulds in nature, and to evaluate how these natural biological phenomena serve as foundational blueprints for bio-digital applications across unconventional computing, bio-electronics, and biotechnology. By contextualizing raw myxomycete biology within applied technological paradigms, this study scientifically contributes by bridging natural non-neuronal behaviors with multidisciplinary engineering while establishing realistic boundaries between empirically established biological evidence and conceptual future models. To achieve this, interdisciplinary peer-reviewed literature was retrieved via Google Scholar and multidisciplinary databases (1970–2026) using Boolean combinations of target organisms ("*P. polycephalum*" OR "Myxomycetes") with domain-specific search terms, including "brain" (or "brainless"), "network", "minimal cognition", "unconventional computing", "Slime Mould Algorithm", "memristor", "secondary metabolites", and "heavy metal". Our synthesis examines the technical scope of the Slime Mould Algorithm (SMA) and its metaheuristic variants, derived from the organism's foraging dynamics. SMA demonstrates strong optimization efficacy across complex industrial and decision-making problems, including power grid load dispatch, photovoltaic system optimization, and medical image segmentation. Furthermore, we assess proof-of-concept integrations of *Physarum* plasmodia into bio-hybrid robotics, alongside the use of spore-based and protoplasmic conductive materials in bio-electronic circuits, memristors, and logic gates. In the biomedical domain, we review specialized secondary metabolites—such as cyclic phosphatidic acid (CPA), Fuligorubin A, and indolo-carbazole alkaloids—which exhibit antiproliferative and antimicrobial activities *in vitro*. Beyond their computational and pharmaceutical relevance, we discuss slime moulds as functional "ecosystem engineers" based on empirical evidence of heavy metal hyperaccumulation (e.g., zinc) and soil microbial modulation. Additionally, we explore conceptual "network imaginaries" where slime mould transport patterns serve as biological analogies for resilient, horizontal governance models and urban infrastructure planning. By evaluating organic adaptive potential alongside silicon-based precision, this review highlights an emerging paradigm of "bio-digital synthesis". While distributed biological mechanisms offer promising frameworks, practical technological implementation remains subject to biological limitations such as physical instability, response latency, and environmental

sensitivity. Ultimately, this review demonstrates that deciphering the non-neuronal processing mechanisms of Myxomycetes not only redefines our understanding of biological intelligence in mycology, but also provides a realistic biological foundation for the future of decentralized bio-digital engineering.

Keywords: *Physarum polycephalum*, slime mould algorithm, bio-digital synthesis, bio-hybrid robotics, anti-tumor metabolites

The Past, Present, and Future of Fungal Computers

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Abstract

Fungal computers represent an emerging class of bio-based computing systems that leverage the intrinsic electrical activity of mycelium networks. This study aims to guide future research directions by reviewing theoretical and experimental models that investigate the information processing, signal transmission, and logical computation capacities of fungi. To this end, a comprehensive literature review was conducted, spanning from foundational studies to recent advancements published up to 2026. Scientific studies demonstrate that fungal mycelia respond to environmental stimuli such as light and temperature through electrical potential fluctuations that functionally resemble neuronal spikes. Furthermore by modulating these bioelectric signals researchers have successfully simulated fundamental logic gates such as AND OR and NOT within fungal tissues. Recent breakthroughs in 2026 have shown that complex logic operations including NAND gates which are the structural building blocks of digital electronics can be effectively modeled using input and output encodings on oyster mushroom (*Pleurotus ostreatus*) networks. Concurrently the literature has expanded to include laboratory scale fully biodegradable analog mycelium substrates alongside Digital Twin software architectures designed for simulating living mycelial behavior in virtual environments. Selecting the optimal fungal strains directly supports the hardware infrastructure of these systems. Characterized by autonomous self healing capabilities and a minimal carbon footprint, fungal computing architectures offer a sustainable alternative for green information technologies. The convergence of computer science with the biotechnological solutions of mycology holds substantial potential for near future applications in smart environmental monitoring sensors, autonomous bio-structures, and hybrid cybernetic systems.

Keywords: Mycelium chips, biorobotics, green computing, digital twin

A Bioinformatics-Based Approach for Interactive PCR-RFLP Assay Design

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Abstract

PCR-RFLP is a widely used, low-cost molecular method for detecting genetic variation. Despite its practical advantages, reliable assay design requires the simultaneous evaluation of primer characteristics, restriction enzyme recognition sites, allele-specific digestion patterns, fragment size resolution, and nearby polymorphisms that may affect interpretation. In this study, a bioinformatics-based workflow was developed to support interactive PCR-RFLP assay design. The workflow allows sequence- or variant-based input and evaluates candidate restriction enzymes according to reference and alternative allele forms. Primer candidates are assessed by product size, melting temperature, allele discrimination capacity, and expected fragment profiles after enzymatic digestion. In cases where a natural restriction site is not available, controlled mismatch-based primer strategies can also be considered. The approach includes visual inspection of the target sequence, primer binding regions, restriction sites, and potential nearby common variants. Variants with relevant population frequency may be flagged to reduce the risk of nonspecific amplification or misinterpretation of digestion patterns. Expected gel fragment profiles are also generated to help select assays with clearly distinguishable bands. This bioinformatics-supported strategy may improve the speed, transparency, and reliability of PCR-RFLP assay planning. It can be useful for molecular genotyping studies, diagnostic assay development, and genetic variation analysis in different biological systems, including mycology-related research.

Keywords: Bioinformatics, PCR-RFLP, primer design, restriction enzyme, genetic variation, molecular genotyping, assay development

Applied and Industrial Mycology

Mycelium-Based Biocomposites: An Evaluation for Passive House Applications

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Abstract

The construction industry has a significant share in energy consumption and greenhouse gas emissions worldwide. This situation accelerates the search to reduce the environmental impact of the materials used in the sector. While traditional insulation materials are effective in reducing energy consumption in buildings, they introduce a high environmental burden on production and waste processes. In this context, bio-based materials stand out as a sustainable alternative. One of the most notable materials in this field is mycelium-based biocomposites. These materials are; It is produced by growing mushroom mycelium on agricultural or industrial wastes. Their ability to be derived from renewable raw materials, biodegradable, produced with low energy, and carbon storage capacity make these materials environmentally appealing. It also coincides with the circular economy approach by transforming waste materials into a functional product. The Passive House standard is one of the most internationally adopted approaches in the design of buildings that provide high energy efficiency. This standard; A well-insulated and airtight building envelope aims to radically reduce the need for heating and cooling through minimised thermal bridging and efficient ventilation systems. The quality of the insulation materials used plays a directly determining role in achieving these goals. This study was conducted to evaluate the usability of mycelium-based biocomposites in Passive House buildings. For this purpose, the relevant literature was comprehensively scanned; the thermal, physical, mechanical and environmental properties of these materials were examined. Research findings indicate that mycelium-based biocomposites have similar thermal conductivity values to many conventional insulation materials, offering a technically usable option in energy-efficient buildings. Their production from renewable resources, biodegradability, low carbon footprint, and capacity to utilize agricultural waste provide significant environmental advantages. On the other hand, challenges such as low moisture resistance, mechanical properties, uncertainties regarding long-term durability, and production standardization still remain to be overcome. In conclusion, mycelium-based biocomposites hold significant potential as a sustainable insulation material for Passive House applications. To realize this potential, there is a need to improve material properties, test long-term performance, and real-scale application studies.

Keywords: Passive house, sustainable building materials, mycelium, biocomposites

From Organic Waste to Mycelium Biomaterials: Designing Industrial Symbiosis

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Abstract

Industrial symbiosis enables the exchange of materials, energy, water, and by-products among different industrial actors, allowing residual outputs from one production process to become inputs for another. Although this approach is increasingly recognized as an important component of circular economy strategies, the integration of organic waste streams into high-value applications remains limited. Compared with inorganic industrial by-products, organic residues pose additional management challenges associated with high moisture content, biodegradability, compositional variability, seasonality, storage, and transportation requirements. Consequently, the industrial reuse of organic waste is often confined to relatively low-value pathways, such as combustion for process energy, use as animal feed, composting, or direct transfer between waste generators and users, while its conversion into high-value materials remains less widely explored. Mycelium-based biomaterials represent a rapidly developing and innovative field of biotechnology that utilizes fungal growth as a biological manufacturing process to transform lignocellulosic and other compatible organic residues into functional materials. Positioned at the intersection of circular economy, biomaterials engineering, and sustainable manufacturing systems, this approach is attracting increasing attention in both academic research and industrial applications. The flexibility of mycelium biocomposite technology as a platform technology enables its adaptation to a broad range of product categories, including packaging, interior design, decorative products, acoustic and insulation solutions, protective components, and various industrial design applications. In this way, organic residues can be diverted from low-value recovery pathways and transformed into value-added products suitable for material-intensive sectors. This study discusses how the biotechnological potential of filamentous fungi, based on their substrate colonization and biomaterial-forming capacities, can be integrated not only into laboratory-scale material production but also into circular systems applicable at the industrial scale. Within this framework, a closed-loop industrial symbiosis model combining mycelium biotechnology with the value-added utilization of organic waste is examined. Rather than transferring a company's organic waste to another sector as a low-value input, the proposed model aims to transform these residues into mycelium-based biomaterials and to utilize the resulting products within the operational, material, or sustainability-related needs of the company from which the waste originated. To examine the industrial applicability of this approach, a pilot-scale implementation model is considered in which organic residues generated from vinegar production processes are evaluated as potential feedstocks for mycelium biomaterial production. Within

this framework, the model aims to establish a closed-loop material flow by converting organic waste into value-added biomaterials through fungal bioconversion processes and subsequently reintegrating the resulting products into the production system. This approach demonstrates the potential of fungal biotechnology to advance industrial symbiosis beyond conventional waste exchange and low-value recovery toward value-added material production. By integrating fungal bioconversion, waste valorization, circular material flows, and industrial material requirements within a single system, a scalable and sustainable model of green industrial collaboration is proposed to support the development of biologically derived alternatives to conventional materials.

Keywords: Mycelium-based biomaterials, industrial symbiosis, organic waste valorization, fungal biotechnology, circular economy, sustainability

A New Future Flourishing in Nature: Mycelium, Sustainable Living, and Human-Centered Education

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Abstract

This presentation addresses making future living spaces more sustainable by steering away from technologycentric approaches without detaching humanity from the biological world to which it belongs. Mycelium is evaluated as one of the fundamental components of this new civilizational paradigm. Natural, local, and recyclable mycelium-based composites demonstrate the potential of sustainable architecture through properties such as carbon sequestration, humidity regulation, thermal insulation, and acoustic damping. To materialize this approach, Anthropia University is being developed in Cholomontas. Integrating production, education, and residential areas within a circular architecture, this hybrid village model offers a self-sufficient settlement proposal featuring mycelium-based systems and a limited population capacity. Taking a critical stance against humanity's alienation from its biological nature toward a mechanical world, this project posits that sustainable living can be established on our native planet using the possibilities offered by nature. The educational pillar of this vision is formed by ROBEK (Ritualized Organic Memory Educational Framework), which centers individuality and physical interaction. Thus, architecture, ecology, and education are conceived as integral components of a new human-centered and nature-harmonious living culture.

Keywords: Mycelium bio-composites, sustainable architecture, Anthropia University, ROBEK, ecological settlement

Breaking the Fungal Shield: A Systematic Review on the Role of Boron Derivatives in Biofilm Inhibition and Dispersal

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Abstract

Historical monuments are of great importance for maintaining cultural continuity; however, they undergo deterioration due to environmental factors, biological organisms, and human-induced causes. Microorganisms particularly microfungi are known to play a significant role in this deterioration process. Biological deterioration caused by microfungi in historical structures occurs when biological agents such as fungi, bacteria, algae, lichens, and mosses adhere to material surfaces and cause structural degradation through chemical, physical, or biomechanical means. This process typically begins with biofilm formation and can eventually lead to cracks, discoloration, salt crystallization, and structural weakening of the building materials. Various methods must be implemented to inhibit the growth of microfungi responsible for this deterioration and to prevent biological degradation in historical structures. Various chemical antifungal agents are used to protect historical artifacts against biological deterioration; however, it is reported that many of these substances contain components that are harmful to the environment, toxic, or pose risks to human health. Consequently, recent studies have aimed to develop alternative protective agents that are more compatible with nature and human health. In this context, boron derivatives stand out due to their broad antimicrobial spectrum, relatively low toxicity, and cost effectiveness. Their use has become widespread across many fields, leveraging the fact that natural boron derivatives are free from harmful substances while possessing antifungal and antibacterial properties. However, there is currently a lack of sufficient literature regarding the effect of boron on the growth of microfungi that cause damage to stone materials and historical structures. The aim of this review is to examine the antifungal effects of various boron compounds against microcolonial fungi which cause biological deterioration in stone and to highlight the diversity of the target fungal species. Studies in the literature have demonstrated that boron compounds are effective for historical monuments and stone structures; they enhance the stone's biological and mechanical strength, improve its durability, and extend its service life. Antifungal efficacy is evaluated through parameters such as enzymatic activity (disruption of cellular components and inhibition of metabolic processes), colony growth inhibition, minimum inhibitory concentrations (MIC), minimum fungicidal concentrations (MFC), spore counts, and microscopic observations. Research aimed at demonstrating the biofilm inhibition and antifungal potential of boron derivatives and investigating their viability as eco-friendly, effective biocide alternatives to combat the microfungal shield on stone structures and in cultural heritage preservation remains limited. This review is expected to provide a detailed examination of studies

concerning the biofilm inhibiting properties of boron derivatives in combating microfungus-induced mycological corrosion, contribute to the development of new approaches, and offer natural, sustainable solution alternatives.

Keywords: antifungal effect, boron derivatives, fungi, fungal shield, fungal biofilm, microcolonial fungi, stone biodeterioration

Antibiofilm Effects of Endophytic Fungal Secondary Metabolites Against *Staphylococcus aureus*

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Abstract

Staphylococcus aureus is a major bacterial pathogen and a leading cause of antimicrobial resistance outbreaks. According to the World Health Organization, drug-resistant infections are projected to cause up to 10 million deaths annually by 2050, further increasing the need for natural alternative compounds to combat the persistent rise in antibiotic resistance. Bacteria within bacterial biofilms are more resistant to antibiotics and immune system responses compared to planktonic bacterial cells. The biofilm structure consists of extracellular polymeric substances such as polysaccharides, proteins, and DNA. Endophytic fungi are microorganisms that live in the tissues of healthy plants without showing any signs of harm or disease. These fungi live in a symbiotic relationship with their host plants and have been shown to produce a wide variety of bioactive compounds that contribute to the overall health and survival of the plant. Recent studies have shown that some endophyte secondary metabolites possess bioactivities with potential applications in medicine, agriculture, and industry. This review aims to understand the effect of endophyte fungal secondary metabolites on *S. aureus* biofilm. Classes of fungal metabolites active against S. aureus include alkaloids, polyketides, terpenoids, peptides, flavonoids, and quinones. Their mechanisms of action include inhibition of cell wall biosynthesis, disruption of biofilm production, and inhibition of nucleic acid or protein synthesis. This review demonstrates that, in line with the “One Health” approach which aims to integrate human, animal, and environmental health, endophytic fungi can play a significant global role in combating antimicrobial resistance.

Keywords: Endophytic fungi, fungal secondary metabolites, antibiofilm activity, *Staphylococcus aureus*

Molecular and Functional Characterization of Ribotoxin-Producing Filamentous Fungi Recovered from Diverse Environmental Sources

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Abstract

Ribotoxins are fungal ribosome-inactivating proteins that induce cytotoxic effects by specifically cleaving the sarcin/ricin domain of 28S rRNA, resulting in inhibition of protein synthesis and apoptosis. This study aimed to investigate ribotoxin-associated gene regions in filamentous fungi, evaluate ribotoxin production, and assess their cytotoxic effects on cancer cell lines. A total of 165 filamentous fungal isolates obtained from agricultural soils, extreme environments, spices, and plant-based animal feed materials were initially identified at the genus level based on phenotypic characteristics. Ribotoxin-associated gene regions were screened by PCR using two specific primer pairs. Eight amplicons showing the expected size for the α-sarcin gene region were selected for sequence analysis, and sequence data were evaluated using Geneious Prime and BLAST analysis against the NCBI GenBank database. Three out of 165 fungal isolates contained ribotoxin-associated gene regions, while the remaining five amplicons showed similarity to hypothetical or uncharacterized proteins. Although the remaining five amplicons showed similarity to hypothetical or uncharacterized proteins, the corresponding strains were included in the subsequent experimental steps. A detailed investigation of their potential association with ribotoxin activity will be performed in future studies. Genotypic identification was performed by ITS1-ITS4 PCR amplification and sequencing, followed by BLAST analysis. The ribonuclease activity of ribotoxin candidates was evaluated by detection of the approximately 400 bp α-fragment released from 28S rRNA. Three fungi exhibited α-fragment release activity (*Aspergillus fumigatus* strain 1, *Aspergillus fumigatus* strain 2 and *Aspergillus restrictus* strain 3). Subsequently, eight fungi were cultured under ribotoxin-inducing conditions, and SDS-PAGE analysis revealed an approximately 18 kDa protein band, corresponding to the expected molecular weight of ribotoxins, only in one isolate (*Aspergillus fumigatus* strain 1), whereas no protein bands within the expected molecular weight range were observed in the remaining seven isolates. Due to resource constraints, complete purification could not be performed. Based on the overall findings, partially purified protein extract containing the putative ribotoxin from *Aspergillus fumigatus* strain 1 were evaluated for cytotoxic activity. The cytotoxic effects were assessed using the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay in PANC-1 and PC-3 cells. *Aspergillus fumigatus* strain 1 exhibited 66.9% and 65% cytotoxicity against PANC-1 and PC-3 cells, respectively. Although previous studies have suggested that ribotoxins may also exert cytotoxic effects on non-malignant cells, evaluation in a healthy cell line was not possible in the present study. This assessment will be addressed in future studies to further characterize the

selectivity of ribotoxin-associated cytotoxicity. These findings indicate the presence of ribotoxin-associated genetic markers and bioactive ribotoxin-producing potential among filamentous fungi.

Keywords: Ribotoxin, fungal toxins, cytotoxicity assay, filamentous fungi

Evaluation of the Biodegradation of Aflatoxin B1 and Aflatoxin G1 by Optimization of *Zygosaccharomyces rouxii* HBB542 Supernatant

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Abstract

Microorganisms are frequently used in aflatoxin biodegradation. This study aimed to optimize the degradation medium conditions of the *Zygosaccharomyces rouxii* HBB542 supernatant, which has been shown in our previous studies to perform aflatoxin degradation at a low level. The goal of this optimization was to achieve higher biodegradation success in aflatoxin degradation. In the optimization of aflatoxin biodegradation studies performed with *Z. rouxii* HBB542 supernatant, Response Surface Methodology (RSM) was used to determine the interaction between three variables (pH, temperature, and metal ions). Aflatoxin analyses were performed using high-performance liquid chromatography (HPLC). As a result of the optimization study, a significant decrease in AFB1 and AFG1 concentrations was observed, especially at pH 9. At pH 9, under all conditions of 20, 35, and 50°C, aflatoxin concentrations decreased rapidly during incubation, and AFB1 and AFG1 were undetectable after 48 hours. At pH 6, a significant decrease in aflatoxin concentrations was observed with increasing temperature; removal was particularly pronounced at 35 and 50°C. Overall, the findings indicate that the aflatoxin removal efficiency of *Z. rouxii* HBB542 supernatant is affected by pH, temperature, and Mg addition, with a significant decrease in aflatoxin concentrations occurring especially under alkaline conditions.

Keywords: Aflatoxin, HPLC, biodegradation

Effects of Mold Fermentations on Food Quality

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Abstract

Mold fermentations have constituted one of the primary biotechnological pillars of traditional food production for centuries; today, they have been repositioned at the center of functional food development and sustainable production strategies. During fermentation processes conducted under controlled environments, the activity of enzymes such as proteases, amylases, and lipases alters the chemical structure of foods, thereby enabling the development of sensory attributes such as aroma, flavor, texture, and color. These transformations are not limited to sensory characteristics; they have also yielded nutritional benefits, including increased protein digestibility, reduction of antinutritional factors, and synthesis of bioactive compounds with high bioavailability. However, achieving these targeted benefits has been linked to the selection of appropriate starter cultures and the precise control of process parameters. Accordingly, the effects of mold fermentations on food quality have been evaluated through the existing literature within the framework of physical, chemical, microbiological, and sensory quality parameters. In particular, quality changes induced by reliable species belonging to the genera *Aspergillus*, *Penicillium*, and *Rhizopus* in different food matrices have been examined, and control strategies against mycotoxin formation risks, the most critical point of the process; have been discussed. Studies have shown that mold fermentations extend beyond prolonging product shelf life and forming natural aroma profiles, offering concrete potential for reducing food waste and valorizing low-value agricultural by-products into value-added foods. Consequently, it has been projected that controlled mold fermentations supported by science-based and reliable production protocols will play a decisive role in enhancing food quality and achieving sustainable production targets.

Keywords: Mold fermentation, food quality, food safety, starter culture, mycotoxin

Macrofungal Diversity, Ecology and Biogeography

Ecological Niche Breadth and Substrate Specialization of Myxomycetes on Anatolian Black Pine (*Pinus nigra* var. *pallasiana*): A Multi-Regional Comparative Analysis

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Abstract

Myxomycetes are sensitive ecological indicators that regulate microbial turnover in forest ecosystems, inhabiting specialized niches shaped by the physical and chemical gradients of host substrates. Anatolian black pine (*Pinus nigra* var. *pallasiana*) serves as an ecologically challenging yet micro-habitatically diverse host, exerting a strong selective filter on microbial communities through its high resin content, tannin density, and cuticle structure. The objective of this study was to comprehensively evaluate the substrate specialization and ecological niche breadth of myxomycete communities across three regional *Pinus nigra* var. *pallasiana* forest reserves in Western Anatolia differing in altitude profiles and stand structure: Eskişehir-Kırka (950–1,150 m a.s.l.), Afyon-Sinanpaşa (1,000–1,200 m a.s.l.), and Afyon-Bayat Köroğlu (1,300–1,500 m a.s.l.) Forests. Aggregating a unified matrix of 213 distinct presence/abundance records, we modeled myxomycete distribution across four primary substrates: live bark of standing trunks, corticolous litter (fallen bark scales), foliicolous litter (pine needles/twigs), and lignicolous (decaying wood). Statistical modeling via Chi-Square (χ^2) test of independence confirmed a highly significant, non-random niche partitioning among the assemblages ($\chi^2 = 217.47$, $p < 0.001$). Shannon-Wiener (H') diversity analysis revealed that the most stable and balanced community clustered on the live bark substrate ($H' = 2.507$), followed by corticolous litter ($H' = 2.335$), lignicolous ($H' = 1.384$), and foliicolous litter ($H' = 1.259$). Taxon-to-Genus (T/C) ratios, calculated to define evolutionary diversification saturation, indicated that the highest taxonomic radiation occurred on lignicolous substrates ($T/C = 2.69$), followed by live bark ($T/C = 1.86$), corticolous litter, and foliicolous litter ($T/C = 1.67$). In chemical analyses, the live bark substrate provided a slightly acidic buffering zone with a mean pH of 5.70, offering a stable micro-environment for specialized taxa (*Physarum luteolum*, *Perichaena vermicularis*) that overcome the host's heavy defense chemistry. Sørensen similarity analysis across substrates demonstrated the highest qualitative overlap between live bark and corticolous litter (42.11%) due to structural ontogeny, while revealing an absolute taxonomic divergence (0.00%) between lignicolous (mean pH 5.45) and foliicolous litter (mean pH 5.54). This radical finding proves that the decaying wood matrix and the acidic needle layers create mutually exclusive ecological worlds. Finally, regional similarity analysis indicated that Kırka and Sinanpaşa—characterized by lower elevation profiles and moderate canopy cover—shared a moderate similarity of 35.62%. Conversely, the Köroğlu Forest pine ecosystem

harbored highly unique assemblages with a low similarity of 14–18%. This taxonomic divergence is driven by Köroğlu's distinct environmental envelope—characterized by higher canopy closure (>70%), elevated relative air/substrate humidity, and a higher altitude profile (1,300–1,500 m a.s.l.)—which buffers ambient thermal stress and creates a microclimatic refugium favoring rare, localized myxomycetes.

Keywords: *Pinus nigra* var. *pallasiana*, Myxomycetes, biodiversity, substrate specialization

Effects of Mycorrhizal Symbiosis on Soil Water Retention and Water Footprint in Agricultural Water Management

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Abstract

Climate change, irregular precipitation patterns, and increasing agricultural water demand require more efficient management of water resources in crop production. The symbiotic association between mycorrhizal fungi and plant roots represents an important biological mechanism that improves plant access to soil water and supports tolerance to drought stress. This review evaluates the potential roles of arbuscular mycorrhizal fungi in maintaining soil moisture, improving soil water retention, enhancing plant water-use efficiency, and reducing the agricultural water footprint. Mycorrhizal hyphae can transport water from fine soil pores that are inaccessible to plant roots. They may also improve soil aggregation, porosity, and water-holding capacity by supporting the formation of glomalin-related soil proteins. Mycorrhizal colonization can influence root architecture, stomatal regulation, osmotic balance, antioxidant defence, and nutrient acquisition, thereby contributing to the maintenance of plant growth under water-limited conditions. These effects may reduce the irrigation water required to obtain a given amount of agricultural product and consequently decrease the blue water footprint per unit of yield. Improved soil moisture conservation and more effective use of precipitation may also increase the contribution of green water to crop production. However, the effectiveness of mycorrhizal applications varies depending on fungal and plant species, soil characteristics, irrigation regime, cultivation system, and environmental conditions. In conclusion, integrating mycorrhizal symbiosis with irrigation optimization, soil health management, and water-footprint assessment provides a nature-based strategy for developing water-efficient and environmentally sustainable agricultural production systems.

Keywords: Arbuscular mycorrhiza, water footprint, water-holding capacity, drought stress, water-use efficiency, sustainable agriculture

Plant Pathology and Agricultural Mycology

Isolation and Microbiological Characterization of Fungal Pathogens Infecting Moroccan Avocado Trees

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Abstract

Root rot is one of the most important diseases affecting avocado (*Persea americana* var. *americana*) production worldwide, leading to reduced plant vigor, decline in productivity, and significant economic losses. Although oomycetes such as *Phytophthora cinnamomi* are commonly associated with avocado root rot, several fungal pathogens may also contribute to disease development. The present study aimed to isolate, identify, and evaluate the pathogenicity of fungal species associated with root rot symptoms in avocado trees cultivated in the Gharb-Chrarda-Beni Hssen region of Morocco. In February 2025, root samples exhibiting typical root rot symptoms were collected from avocado orchards located in Souk El Had, Ouled Jelloul. Symptomatic root tissues were surface-sterilized and cultured on appropriate growth media to obtain fungal isolates. The recovered fungi were purified and identified based on their macroscopic and microscopic morphological characteristics. Two fungal genera were consistently isolated from diseased roots and were identified as *Fusarium* sp. and *Alternaria* sp. To determine their pathogenic potential, pathogenicity tests were conducted under controlled conditions using young avocado plants. The fungal isolates were inoculated onto healthy plants, which were subsequently monitored for symptom development. Disease severity was assessed by evaluating root discoloration, tissue necrosis, and overall plant health. The inoculated plants developed symptoms similar to those observed in the field, confirming the pathogenic nature of the isolates. The findings indicate that *Fusarium* sp. and *Alternaria* sp. are associated with avocado root rot in the studied area and may contribute to disease development. These results provide valuable information for understanding the etiology of avocado root diseases and may support the development of effective disease management strategies in Moroccan avocado orchards.

Keywords: Root rot, avocado, Oomycetes, pathogenicity, fungal species

Seasonal Microfungal Biodiversity Assessment of Fallow Soils in Turkey's Hop-Growing Region

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Abstract

Humulus lupulus L. (hops), naturally distributed across Europe, North America, and Asia, is cultivated in Turkey only in Pazaryeri (Bilecik). According to the Turkish Ministry of Agriculture and Forestry's Agricultural Investment Guide (2024) and the 2024–2028 BEBKA Regional Strategy Plan, hops are classified as "selected field crops," with expansion of production into new areas identified as a strategic goal. In line with these priorities, Pazaryeri—the sole region of hop cultivation in Turkey—was selected for investigation. To assess the potential risks and opportunities for hop production, fallow fields within the district boundaries were surveyed for microfungal biodiversity, focusing on potential plant pathogens. Soil samples were collected seasonally (February, May, August, November 2025) from northern, southern, eastern, and western subregions following the LUCAS protocol, and physicochemical properties were analyzed. Fungal isolation was performed using the Dilution Plate Method on selective media (SNA, SFA + Rose Bengal, PDA + Rose Bengal, all supplemented with streptomycin) incubated at 25 °C for 14 days. A total of 231 isolates were recovered: 81 in winter (35%), 68 in spring (29%), 32 in summer (14%), and 50 in autumn (22%). To date, 153 isolates have been identified through cultural characterization and multilocus gene analyses (ITS, RPB2, EF). The isolates represented a broad taxonomic spectrum, with *Fusarium* species dominating (~30%). *Fusarium oxysporum* was the most frequent species (27 isolates, 17.6%), followed by *Clonostachys rosea* (13 isolates, 8.5%), *Trichoderma harzianum* (8 isolates, 5.2%), and Phoma herbarum (6 isolates, 3.9%). Other taxa such as *Fusarium solani*, *Gongronella butleri*, *Purpureocillium lilacinum*, and *Trichoderma velutinum* were moderately represented (5 isolates each, 3.3%), while more than 30 taxa were recorded only once, underscoring high microfungal diversity. Overall, the coexistence of pathogenic and antagonistic fungi highlights the dual role of soil biodiversity. Disease pressure is evident, yet ecological management practices that enhance beneficial fungi can strengthen agrobiodiversity, improve soil resilience, and support sustainable hop production systems in Turkey.

Keywords: *Humulus lupulus*, seasonal soil fungi, agrobiodiversity, Pazaryeri

PCR-Based Detection of *Macrophomina phaseolina* Causing Charcoal Rot of Sunflower

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Abstract

The fungus *Macrophomina phaseolina* can infect great number of hosts. As one of these hosts; sunflower (*Helianthus annuus*) is an indispensable oilseed crop and the primary source of vegetable oil production in Türkiye. Unfortunately, in recent years, climate change and increased drought resulted in the spread of soil-borne fungal pathogens and eventually led to significant yield losses in sunflower production. One of the major factors of these losses, *M. phaseolina* is the causal agent of charcoal rot disease in sunflowers, so the diagnosis and control of the pathogen have become crucial. The pathogen is particularly aggressive under high-temperature and drought conditions and can survive in the soil for many years in the form of microsclerotia. Since disease symptoms typically appear during the later stages of plant development, early detection is often difficult, resulting in ineffective disease management and yield losses of up to 50–90%. To overcome these challenges, PCR-based molecular diagnostic techniques provide rapid, sensitive, and specific detection of plant pathogens. Polymerase chain reaction (PCR) is a widely used and reliable molecular technique allowing accurate and early detection of the pathogen in infected plant tissues. Compared with conventional diagnostic methods, PCR provides faster results with high sensitivity and specificity. The internal transcribed spacer (ITS) region has been widely used as a molecular marker for the detection of fungal plant pathogens. Previous studies have demonstrated that the ITS region has limited discriminatory power for species-level detection of *M. phaseolina*. Therefore, in this study, additional candidate genome regions suitable for the molecular detection of *M. phaseolina* were investigated (TEF-1α, β tubulin, Calmodulin, Actin) and species-specific primers were designed according to these regions. PCR conditions were optimized for all primer pairs and their specificity were tested with different fungal plant pathogens (*P. orizae*, PT. varior, PT. raise1, *C. satirus*, Pamq, Ustilago, *F. teres*, *P. strifomis* YR-27, *F. culmorum*, *F. graminearum*). The main purpose of the study was to improve the reliability of the molecular detection of M. phaseolina by developing additional species-specific primers. Among the designed primer sets, those targeting the TEF-1α and Actin loci of *M. phaseolina* exhibited the highest discriminatory power. The PCR products obtained with the designed primers were run on agarose gel electrophoresis and visualised under UV light. None of the other tested pathogens produced bands at 599 bp for TEF-1α and 213 bp for Actin whereas *M. phaseolina* did. In conclusion, discriminatory genomic regions suitable for the molecular detection of *M. phaseolina* were identified, and species-specific primer sets were successfully developed. These findings have the potential to contribute to the development of early

molecular detection systems for agricultural applications, thereby helping to reduce sunflower yield losses in the Thrace Region and supporting sustainable crop production.

Keywords: *Macrophomina phaseolina*, phytopathogenic fungi, fungal diagnostics, early detection, PCR, charcoal rot, multi-loci analysis

New Locality Record of *Puccinia marrubii* (Pucciniaceae) on *Marrubium globosum* subsp. *globosum* (Lamiaceae) from Antalya, Türkiye

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Abstract

Puccinia marrubii Kabaktepe, Mutlu, Karakuş & Akata is the first *Puccinia* species described within the genus *Marrubium* (Lamiaceae). It was identified as a new rust fungus species in 2016, based on specimens collected from *Marrubium globosum* subsp. *globosum* Montbret & Aucher ex Benth. in the Niğde and Malatya provinces of Türkiye. During field surveys conducted in the summer and September of 2025, rust-infected specimens of *M. globosum* subsp. *globosum* were collected from the Akseki district of Antalya province. Telial sori were observed on the stems, petioles, and lower leaf surfaces of the host plant. The collected samples were examined using stereo and light microscopy. Species identification was based on the comparison of macro- and micromorphological features with the species' original description and relevant literature. The examined specimens were identified as *P. marrubii*, and their morphological characteristics were found to be consistent with the original description of the species. This finding establishes a new locality record for *P. marrubii* and extends its known distribution in Türkiye to Antalya province. The macro- and micromorphological characteristics of the examined specimens are presented along with macro- and microphotographs.

Keywords: *Puccinia marrubii, Marrubium globosum* subsp. *globosum*, rust fungi, telial sori, new locality record, Antalya

Investigation of the Utilization Possibilities of Corn Cobs in *Pleurotus ostreatus* Cultivation

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Abstract

This study aimed to determine the effects of different substrate mixtures on mycelial growth and yield performance in the cultivation of *Pleurotus ostreatus*. In the study, wheat straw and wheat bran were used as the main substrates, and different proportions of corn cob powder,corn cob pieces, and whole corn cobs were added to create different experimental groups. The spawn mycelia used in the experiments were prepared by multiplying pure cultures on Potato Dextrose Agar (PDA) medium and developing mother cultures on wheat grains. The prepared substrates were sterilized and made suitable for mycelial inoculation under appropriate moisture and pH conditions. In the study, wheat straw + wheat bran mixtures, groups containing different ratios of corn cob powder, and mixtures containing corn cob pieces and whole corn cobs were compared. The substrates were inoculated with 5-7% spawn mycelium and incubated under suitable temperature and humidity conditions. Mycelial growth period, primordium formation, and mushroom yield were evaluated. The mushroom yield trials for the study are ongoing. The study found that the addition of corn pieces and the use of whole corn cobs positively impacted mycelial growth in the compost to varying degrees compared to the control group.

Keywords: *Pleurotus ostreatus*, substrate, wheat straw, wheat bran, corn cob, mycelial growth, mushroom yield.

Soil Microfungi as Biocontrol and Plant Growth-Promoting Agents : An Agroecological Approach

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Abstract

The environmental impacts of synthetic fungicides and chemical fertilizers, together with the emergence of resistant phytopathogens, have increased interest in environmentally friendly alternatives for sustainable crop production. In this study, the biocontrol potential and plant growth-promoting properties of seven filamentous fungi belonging to the genera *Trichoderma, Mucor, Penicillium, Arthrinium*, and *Aspergillus*, isolated from agricultural soils in Çanakkale and Edirne (Türkiye), were investigated. The antagonistic activities against the phytopathogenic fungi *Botrytis cinerea* and *Trichothecium roseum* were evaluated using dual culture and sealed double-dish assays. Additionally, in vitro plant growth-promoting traits, including phosphate solubilization, indole-3-acetic acid (IAA) production, extracellular protease activity, hydrogen cyanide (HCN) production, and siderophore production, were evaluated. Among the tested isolates, *Trichoderma* sp. showed the strongest antagonistic activity in dual culture, inhibiting the mycelial growth of *B. cinerea* and *T. roseum* by 63.70% and 51.09%, respectively. In sealed double-dish assays, *Mucor* sp. exhibited the highest inhibition against *B. cinerea* (43.33%), whereas Trichoderma sp. was most effective against *T. roseum* (41.46%). Overall, a *Penicillium* isolate exhibited the broadest spectrum of plant growth-promoting traits, whereas *Trichoderma* sp. showed the strongest antagonistic activity against the tested phytopathogens.

This study was supported by the Scientific and Technological Research Council of Türkiye (TÜBİTAK) under the 2209-A University Students Research Projects Support Program.

Keywords: Antagonistic fungi, plant growth-promoting fungi, biocontrol, phytopathogens

Fungal Symbioses: Mycorrhizae, Lichens and Associated Interactions

Biomonitoring of Atmospheric Microplastics in Istanbul's Green Areas Using *Xanthoria parietina*: A Multi-Analytical Approach

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Abstract

Microplastics, accumulating across atmospheric, aquatic, and terrestrial ecosystems due to anthropogenic activities, pose multidimensional ecological risks to biodiversity, soil integrity, and trophic chains. Driven by the necessity to monitor this crisis, this study utilizes the common epiphytic lichen *Xanthoria parietina* (L.) Th.Fr.—renowned for its pollutant entrapment capacity—as a biomonitor to investigate atmospheric microplastic deposition on the European side of Istanbul. Samples collected from 11 stations in green areas were analyzed via the Nile Red protocol under fluorescence microscopy, with quantitative data extracted using ImageJ software. The findings revealed a broad spectrum of microplastic densities compared to the reference lichen sample. Pollution pressure peaked in urban areas such as Emirgan Grove and Göktürk Pond Nature Park, while remaining limited in peripheral locations like Çatalca National Park and Paşaeli Recreation Area. SEM-EDS analyses confirmed the polymeric nature of the particles through high Carbon (C) and Oxygen (O) ratios, while uncovering industrial plastic additives (Ti, F, P, Ba) alongside traffic-derived metallic residues (Fe, Cu, Ni). Based on FTIR spectroscopic data of the investigated locations were categorized into three distinct classes based on component densities: High-Density Organic Matrices: Taksim Gezi Park, Alparslan Türkeş Woods, Emirgan Woods, Florya Atatürk Forest, and Gülhane Park exhibited intact cellular matrix structures with high-quality spectral profiles rich in proteins, functional groups (O-H, C-H, Amide I/II), heteropolymeric biomass components, and moisture. Mixed/Environmentally Influenced Matrices: Göktürk Nature Park presented a hybrid structure, displaying a stable biological fingerprint alongside significant surface accumulation of anthropogenic or natural silicates and dust. Inorganic and Mineral-Dominated Phases: Paşaeli Park, Çatalca National Park, and İnceğiz Caves showed attenuated organic signals, heavily dominated by inorganic phases, mineral dust, and silicates.

Supported by Marmara University (BAP Project: FYL-2024-11423), this study contributes to the literature by documenting atmospheric MP pollution via biomonitors, establishing a foundation for scientific awareness.

Keywords: Air pollution, airborne microplastics, biomonitoring, environment, lichen

Antifungal Activity of Acetone Extracts from Lichens Incorporated into PVA-Based Polymeric Membranes

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Abstract

Lichens are a rich source of bioactive secondary metabolites with recognized antimicrobial potential. In this study, acetone extracts obtained from the epiphytic lichen species *Ramalina farinacea*, *Evernia prunastri*, *Usnea florida*, and *Pseudevernia furfuracea* were evaluated for antifungal activity and their integration into functional polymeric membranes. Dried lichen thalli were extracted with acetone, and crude extracts were tested against *Candida albicans* ATCC 10231, *C. tropicalis* KUEN 1021, *C. glabrata* ATCC 90030 using agar diffusion and minimum inhibitory concentration (MIC) assays. Minimum fungicidal concentration (MFC) values of the lichen extracts againsts the three *Candida* species were also determinated. The extracts were subsequently incorporated into polyvinyl alcohol (PVA) matrices via solvent casting to produce bioactive membranes. The same antifungal tests were performed on the formed PVA membranes containing lichen extracts in order to assess the candicidal activity. Membrane morphology and homogeneity were examined to confirm effective dispersion of the extracts within the polymer matrix. PVA membranes containing lichen extracts retained antimicrobial functionality, suggesting sustained release of active compounds. Among the lichen extracts *Ramalina farinacea* formed inhibition zones againsts *C. albicans* and *C. tropicalis* (13.09 mm; 10.95 mm respectively). Additionally, *Evernia prunastri* extract was effective against *C. albicans*, creating an inhibition zone of 9.28 mm. All lichen extracts were found to be effective against *C. albicans* and *C. glabrata*. PVA, containing parietin and *Pseudevernia furfuracea* lichen, was found to be effective against all *Candida* strains. These results demonstrate that acetone extracts from selected lichens can be successfully incorporated into PVA-based systems, highlighting their potential for use in antifungal coatings and active packaging applications. Consequently the results of the present study shows that acetone extracts of lichens and also their PVA-based polymeric membranes could be used in future study in wound healing. This study was supported by Marmara University Scientific Research Projects Unit (BAPKO) under the number FDK-12165.

Keywords: Lichens, antifungal activity, polyvinyl alcohol (PVA), polymeric membranes, bioactive materials

Fungal Natural Products and Chemical Ecology

Isolation, Structural Elucidation, and Biological Evaluation of Secondary Metabolites from *Aspergillus* sp. isolate 1352

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Abstract

Aspergillus-derived secondary metabolites are known for their broad biological activity, making them a significant focus for the development of new therapeutic agents.The aim of this study is to investigate the metabolic profile of *Aspergillus* sp. isolate 1352 and to evaluate the biological activities of the secondary metabolites produced in solid culture. In this study, the cultivation and production of *Aspergillus* sp. isolate 1352, which exhibits a distinct HPLC-DAD profile and produces bioactive secondary metabolites in solid culture (Yeast Extract Sucrose Agar), was conducted. Crude extracts, exhibiting potential anticancer, antibacterial, and antifungal activities, were fractionated using various extraction and chromatographic techniques, followed by an evaluation of their biological profiles. Following the screening of active fractions, the secondary metabolites were purified and characterized using a combination of techniques, including silica gel and C-18 reversed-phase chromatography, precipitation, HPLC-DAD, flash chromatography, TLC, semi-preparative HPLC, NMR, and LC-MS. In this process, the molecules responsible for the observed activity were successfully isolated. As a result, Aurosperone F, Rubrofusarin, Rubrafusarin B, Flavasperone, Fonsecin, and Fonsecin B were identified from *Aspergillus* sp. isolate 1352. Fonsecin showed antifungal activity against *Mucor hiemalis* DSM 2656 and also Fonsecin B exhibited antifungal activity against *Candida albicans* DSM 1665, *Mucor hiemalis* DSM 2656, and *Aspergillus clavatus* DSM 816. In conclusion, the bioactivity-guided isolation and characterization of *Aspergillus* sp. isolate 1352 metabolites, particularly Fonsecin and Fonsecin B, reveal significant potential for future antifungal applications.

The authors would like to thank The Scientific and Technological Reserch Council of Turkey (TUBITAK) under the grant number 117Z111 for financial support of this study.

Keywords: *Aspergillus*, secondary metabolites, Fonsecin, antifungal activity

Bioactivity-Guided Isolation and Characterization of *Aspergillus brasiliensis* Metabolites: Assessment of Biological Activities

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Abstract

Aspergillus section *Nigri* (Black Aspergilli) are known to produce secondary metabolites with diverse structures and significant anticancer and antimicrobial potential. This study aims to isolate and characterize bioactive secondary metabolites from *Aspergillus brasiliensis* and assess their anticancer and antimicrobial potential. *Aspergillus brasiliensis* was identified through a polyphasic approach, incorporating morphology, multi-locus sequencing (ITS, beta-tubulin and calmodulin genes), and extrolite profiling, within the scope of another project. Preparative liquid culture fermentations were conducted in Yeast Extract Sucrose (YES) broth under selected conditions, including a C/N ratio of 32, a temperature of 25 °C, a pH of 6, and an agitation speed of 210 rpm. The liquid culture fermentation was filtered to separate the supernatant and mycelium. Subsequently, the crude extracts obtained from both fractions were purified and characterized using High-Performance Liquid Chromatography (HPLC-DAD), Flash Chromatography, Thin Layer Chromatography (TLC), Semi-Preparative HPLC, and Nuclear Magnetic Resonance (NMR) spectroscopy. As a result, seven pure secondary metabolites were isolated from *Aspergillus brasiliensis*: six from the supernatant and one from the mycelium. NMR analysis revealed these compounds to be Malformin A1, A3, B3, and B4; Brasenol A1; and two itaconic acid derivatives (9-HHIA and 10-HHIA). Apoptotic assays revealed that Malformin A3 exhibits selective caspase activity. Furthermore, this metabolite demonstrated significant antifungal effects against *Mucor hiemalis* DSM 2656 and *Aspergillus clavatus* DSM 816, as well as antibacterial activity against *Staphylococcus aureus* ATCC 6538. In conclusion, the metabolites of *Aspergillus brasiliensis*, particularly Malformin A3, exhibit significant anti-cancer and antimicrobial potential, and these compounds are promising candidates for further preclinical studies.

The authors would like to thank The Scientific and Technological Reserch Council of Turkey (TUBITAK) under the grant number 117Z111 for financial support of this study.

Keywords: *Aspergillus brasiliensis*, secondary metabolites, bioactivity-guided isolation, structural characterization, biological potential

POSTER PRESENTATIONS

Medical and Veterinary Mycology

Aquatic Fungal Pathogens in Experimental Animal Models

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Abstract

Aquatic mycological diseases represent a major and increasingly recognized threat to experimental aquatic animal research globally, affecting the reliability of biomedical and veterinary studies that rely on teleost fish and amphibian models. This review systematically characterizes the most frequently encountered aquatic fungal taxa in experimental animal settings, encompassing true fungi and oomycetes (fungal-like *Stramenopila*). The principal pathogens examined are *Saprolegnia parasitica* — causative agent of saprolegniosis and the most economically damaging aquatic oomycete disease worldwide — Batrachochytrium dendrobatidis (Bd), the chytrid fungus implicated in the decline of over 500 amphibian species and associated with mortality risk in *Xenopus* laboratory colonies, *Aphanomyces invadans*, etiological agent of Epizootic Ulcerative Syndrome listed by the World Organisation for Animal Health (WOAH), *Ichthyophonus hoferi*, a systemic pathogen at the animal-fungal taxonomic boundary, *Fusarium solani*, associated with mortalities in marine reptile eggs and opportunistic fish infections, and *Exophiala angulospora,* causing granulomatous systemic mycosis in marine teleosts. The pathogenic mechanisms, experimental transmission models, and histopathological features of each taxon are critically reviewed. A central finding is that therapeutic options are severely constrained: oomycetes are intrinsically resistant to conventional ergosterol-targeting antifungal drugs due to their cellulose-based cell walls, while the most effective chemical agents, namely malachite green and formalin, are banned or restricted in most jurisdictions due to carcinogenicity, teratogenicity, and environmental toxicity. This therapeutic vacuum necessitates a paradigm shift from treatment toward prevention. Evidence-based prevention frameworks applicable to experimental aquatic animal facilities are therefore systematically evaluated, including biosecurity protocols, water quality optimization, stress reduction, biological control using antagonistic *Pseudomonas* strains, phytogenic and probiotic alternatives, and molecular surveillance via environmental DNA sampling. Prevention is concluded to be the primary and most reliable defense strategy against aquatic fungal pathogens in experimental settings pending the development of effective alternative therapeutics.

Keywords: Aquatic mycology, Saprolegniosis, chytridiomycosis, experimental animal model, antifungal prevention, biosecurity

Preparation of selenium nanoparticles in plant extract substrates and investigation of antifungal properties and anticancer effects of cell lines

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Abstract

Species of *Aspergillus* and Mucorales are among the most important opportunistic fungal pathogens, causing a substantial burden of invasive fungal infections worldwide. The emergence of antifungal resistance among these organisms has become an increasing concern, limiting the effectiveness of currently available therapeutic options. This study evaluated the green synthesis of selenium nanoparticles (SeNPs) using *Nymphaea alba* L. extract as a natural reducing and stabilizing agent and assessed their antifungal and anticancer activities. The synthesized SeNPs were characterized using various analytical techniques and demonstrated spherical morphology, high stability, and particle sizes ranging from approximately 15 to 50 nm. The antifungal activity of SeNPs was investigated against 16 clinical and reference fungal isolates, including *A. flavus, A. fumigatus*, and *Rhizopus arrhizus*. Antifungal susceptibility testing was performed using the CLSI M38-A2 broth microdilution method. The minimum inhibitory concentration (MIC) values ranged from 125 to 1600 µg/mL for *A. flavus*, 200 to 1600 µg/mL for *A. fumigatus*, and 5.62 to 250 µg/mL for *R. arrhizus*. The strongest antifungal activity was observed against *R. arrhizus*, a major etiological agent of mucormycosis. Furthermore, SeNPs exhibited significant anticancer activity against multiple cancer cell lines, highlighting their potential therapeutic applications. The findings suggest that plant-mediated selenium nanoparticles represent a promising biogenic nanomaterial with potential applications as both antifungal and anticancer agents. However, additional studies are needed to further evaluate their efficacy against clinical isolates of *A. flavus* and *A. fumigatus* and to validate their safety and effectiveness in vivo.

Keywords: *Aspergillus*, Mucorales, selenium, nanoparticles, antifungal activity

Bioactive Components of *Auricularia auricula-judae* (Bull.) Quél. and Its Potential in Wound Healing: A Current Review

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Abstract

Auricularia auricula-judae (jelly ear mushroom) is a widely cultivated edible fungus with well documented pharmacological properties. Due to its high nutritional valueand the biologically active compounds it contains, it is of interest for functional food and biomedical applications. Recent researches have reported that *A. auricula-judae* and related *Auricularia* species are rich in polysaccharides, proteins, phenolic compounds and other bioactive metabolites, and these components can exhibit various biological effects, including antioxidant, antitumor, hypolipidemic andanticoagulant/antithrombotic activities. In recent years, the effects of *A. auricula-judae* polysaccharides (AAP) on wound healing, in particular, have attracted attention. In this review, the pharmacological properties of the bioactive components of *A. auricula-judae*, mainly polysaccharides, have been considered; in particular, their potential effects on wound healing have been evaluated. Experimental studies show that AAP extracts contribute to thefundamental cellular processes of wound healing by promoting the proliferation and migration of fibroblasts and keratinocytes. In addition to cell culture studies, in vivo experiments have shown that AAP application accelerates the wound healing process and promotes tissue regeneration. Since the current evidence is largely based on cell culture andanimal models, further studies are needed to elucidate the mechanisms of action of AAP in detail, standardize the extracts, determine their safety profiles, and evaluate theirclinical efficacy. As a result, *A. auricula-judae* can be considered as a promising natural biological resource, especially worthy of research in the field of wound healing and tissue regeneration through polysaccharide fractions.

Keywords: *Auricularia auricula-judae*, medicinal mushroom, wound healing, polysaccharides

Evaluation of Antifungal Potential of *Morinda citrifolia* L. (Noni) Against Dermatophytes

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Abstract

Morinda citrifolia L., commonly known as Noni, is a small, tropical, evergreen plant belonging to the Rubiaceae family and widely cultivated in tropical regions. *M. citrifolia* has attracted attention in traditional medicine for its antimicrobial, anti-inflammatory, and antifungal effects. Acids, alcohols, flavonoids, anthraquinones, esters and other minor compounds are found in *M. citrifolia.*Also leaves,seeds, bark, roots, flowers, stems and fruits of the *M. citrifolia* have been used in the treatment of some diseases such as cancer,inflammation, tumor, diabetes, arthritics, hypertension, asthma, pain and topical infections. Dermatophytes infect keratinized tissues (skin, nails, and hair), causing symptoms such as itching, redness, scaling, and hair loss. The zoonotic potential of these infections poses a serious public health risk to both animals and humans. Various studies on the antifungal effects of *M. citrifolia* have highlighted the fruit's potential in the treatment of fungal infections. These studies have emphasized the fruit's antifungal effect on microorganisms that target keratinized tissues, such as dermatophytes. It has been reported that the phenolic compounds in the noni fruit exhibit antifungal activity by damaging the structure of the fungal cell membrane. The purpose of this review is to highlight the effects of *M. citrifolia* against dermatophyte infections based on the results of studies conducted at various times to date and to evaluate its potential for use as a new antifungal agent. Current evidence suggests that nitric oxide, an extract of *M. citrifolia*, possesses promising antidermatophytic potential, particularly against the species *Trichophyton mentagrophytes.* Thus, this approach not only offers an innovative solution to problems such as resistance and side effects commonly encountered in traditional antifungal treatments but also contributes to the development of a natural alternative as an antifungal agent.

Keywords: *Morinda citrifolia* L., dermatophytes, antifungal potential, therapeutic effects

Fungal Systematics, Taxonomy and Nomenclature

Phylogenetic analyses, morphological characteristics, and host specificity of a smut fungus on *Carex glacialis* (Cyperaceae) reveal a new species, *Anthracoidea glacialis* (Anthracoideaceae, Ustilaginales)

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Abstract

Anthracoidea were considered to be restricted to host plants belonging to the same or closely related sections of Carex, but our intensive studies of Anthracoidea specimens on different host species show that the specialization is much narrower than previously assumed. *Carex glacialis* is a circumpolar sedge, distributed in Alaska, Canada, Greenland, Iceland, Fennoscandia, and Russia. It belongs to Carex sect. Acrocystis. In the past, the Anthracoidea specimens on *Carex glacialis* were referred to A. rupestris. The present molecular study demonstrated that Anthracoidea rupestris is specialized on *Carex rupestris* while Anthracoidea on *Carex glacialis* is a distinct species. As a result of this study, a new species, *Anthracoidea glacialis* on *Carex glacialis*, is proposed. Three Anthracoidea species have been previously reported on carices in C. sect. Acrocystis: Anthracoidea caricis, A. caryophylleae, and A. caricis-reznicekii. Anthracoidea caricis in its former traditional circumscription was a collective species, while in its current circumscription it is a fungus distributed on *Carex pilulifera*. For the distinctness of *A. caricis* on *C. pilulifera* from *A. glacialis*, there is molecular phylogenetic evidence. The hosts of *A. caryophylleae* are currently reduced to carices in Carex sect. Mitratae. In addition, there is molecular phylogenetic evidence for the distinctness of *A. caryophylleae* from *A. glacialis*. Anthracoidea caricis-reznicekii differs from *A. glacialis* by the presence of spores with conspicuous, often numerous (up to 8) protuberances in contrast to the fewer and less conspicuous protuberances of *A. glacialis*, and by having a spore wall with higher warts (0.2–0.5 µm high versus up to 0.15 µm high for *A. glacialis*). Anthracoidea glacialis differs from all other species in this genus based on molecular data and/or by specialization on *Carex glacialis*. Anthracoidea glacialis is a circumpolar species distributed in North America (Canada – Nunavut, Manitoba, Ontario, and Quebec; and Greenland), Europe (Norway, Sweden, and Finland), and Asia (Arctic Russia).

This study was supported by the Bulgarian National Science Fund (Grant no. KP-06-N91/6/03.12.2025).

Keywords: Greenland, phylogeny, smut fungi

Artificial Intelligence, Image Analyses and Digital Mycology

Benchmarking Evo 2 Against Experimental Fks1 Fitness Landscapes of Echinocandin Resistance

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Abstract

Antifungal resistance is becoming an important problem in medical mycology. However, resistance mutations do not all work in the same way. Some mutations reduce normal cellular fitness, while others may preserve fitness but change how the cell responds to antifungal treatment. This difference is important for genomic foundation models such as Evo 2. Evo 2 learns patterns and constraints directly from DNA sequences, but it does not receive information about antifungal drugs during prediction. Our main hypothesis is that Evo 2 will predict the general fitness effects of Fks1 mutations better than their drug-specific effects under echinocandin treatment. To test this hypothesis, we use a published deep mutational scanning dataset of the Saccharomyces cerevisiae Fks1 beta-1,3-glucan synthase, which is the main target of echinocandin antifungal drugs. The dataset covers the three main resistance hotspot regions of Fks1. In the published study, 465 single mutants were confidently classified under echinocandin conditions. The mutations were produced using saturation mutagenesis and introduced into the native FKS1 locus. Their effects were measured by following changes in variant frequency during competitive growth experiments. An important advantage of this dataset is that the same mutations were tested both without antifungal treatment and under three clinically important echinocandins: anidulafungin, caspofungin, and micafungin. This allows us to compare the drug-free fitness effect of each mutation with its effect during antifungal treatment. The available experimental data, sequencing resources, and analysis code also allow the study to be reproduced and checked. For the computational analysis, experimentally tested nucleotide variants are placed in their original genomic sequence context and evaluated with Evo 2 without task-specific training. For each variant, we calculate the change in sequence likelihood between the wild-type and mutant sequence. These Evo 2 scores are then compared with the experimentally measured selection coefficients. The control condition and each antifungal treatment are analyzed separately. We also use the matched control data to distinguish general fitness effects from drug-associated selective effects. If Evo 2 scores show a stronger relationship with fitness in the control condition than with effects observed specifically during drug treatment, this would suggest that the model mainly captures general biological and evolutionary constraints. In contrast, if Evo 2 also predicts effects under echinocandin treatment, this would suggest that genomic sequence context may contain information related to antifungal resistance beyond normal cellular fitness. Because Evo 2 is an autoregressive DNA model, we also test whether sequence

orientation and genomic context size affect the results. These analyses are defined before evaluating their relationship with the Fks1 experimental phenotypes, to avoid selecting conditions only because they produce better performance. This study aims to determine what Evo 2 can and cannot capture about experimentally measured antifungal resistance. The results may provide a basis for future studies using additional fungal resistance genes and different resistance mechanisms.

Keywords: antifungal resistance, genomic foundation model, deep mutational scanning, fungal genomics, variant effect prediction

Applied and Industrial Mycology

Mycelial Pellet Morphology and Pigmentation of Selected *Penicillium* and *Talaromyces* Species Vary With Culture Medium Mineral Composition

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Abstract

Penicillium and *Talaromyces* species produce secondary metabolites and pigments that respond to nutrient availability, yet how medium mineral composition shapes mycelial pellet phenotype across multiple species has not been systematically compared in liquid culture. Eight standard strains, *Penicillium citrinum*, *Penicillium glabrum*, *Penicillium griseofulvum*, *Penicillium oxalicum*, *Talaromyces purpurogenum*, *Talaromyces funiculosus*, *Talaromyces islandicus*, and *Talaromyces verruculosus*, were grown on four liquid media in both original and modified compositions. Original formulations contained a trace metal solution (zinc sulfate and copper sulfate); modified versions replaced or supplemented this with a Czapek mineral concentrate (sodium nitrate, potassium chloride, magnesium sulfate, and ferrous sulfate). The four liquid media represented a range of carbon source complexity: malt extract-peptone-glucose broth, Czapek Yeast broth, malt extract broth, and a minimal sucrose-based basal broth. All strains exhibited medium-dependent differences in mycelial pellet morphology and pigmentation. *Talaromyces purpurogenum* showed the sharpest contrast, producing bright red mycelial pellets on several media but near-black mycelial pellets on Czapek Yeast broth. *Talaromyces islandicus* and *Talaromyces verruculosus* generated intensely colored diffusible pigments that varied markedly between original and modified formulations. Growth was most restricted on the minimal sucrose-based liquid medium across all strains. These phenotypic differences suggest underlying variation in secondary metabolite biosynthesis. Culture medium supernatants from both formulation types will therefore be subjected to comparative bioactivity profiling, including antimicrobial, antioxidant, antidiabetic, anti-inflammatory, anticholinergic, and cytotoxic assays. Supernatants exhibiting differential activity will constitute a screening library for subsequent chemical characterization aimed at identifying bioactive compounds with therapeutic potential.

Keywords: *Penicillium, Talaromyces*, mycelial pellet morphology, pigmentation, culture medium

Catechol Biodegradation and Antioxidant Defense Responses in Halotolerant Filamentous Fungi

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Abstract

The increasing release of xenobiotic compounds into the environment poses a serious ecological challenge due to their persistence and toxicity. Among these pollutants, phenolic compounds such as catechol (1,2-dihydroxybenzene) are common contaminants of industrial effluents and can adversely affect aquatic and terrestrial ecosystems. Consequently, the development of sustainable methods for their removal has become a priority, with microbial biodegradation representing one of the most effective and environmentally friendly approaches for the detoxification of phenol-contaminated environments. Among the diverse microbial degraders, halotolerant filamentous fungi have attracted increasing interest because of their ability to withstand multiple environmental stresses while maintaining high metabolic activity. The present study aimed to investigate the metabolic capacity and antioxidant defense mechanisms of saline-adapted filamentous fungi upon exposure to phenolic compounds. Specifically, it examines the ability of fungal mycelia to degrade catechol (1,2-dihydroxybenzene) through the secretion of extracellular polyphenol oxidases, including laccases and tyrosinases. The biodegradation process and the resulting detoxification of the growth medium were systematically monitored. Simultaneously, the intracellular oxidative stress induced in the fungal hyphae was evaluated by assessing the activities of the key antioxidant enzymes superoxide dismutase (SOD) and catalase. The results demonstrate a strong correlation between the extent of catechol degradation and the coordinated activation of the enzymatic antioxidant defense system in fungal cells. These findings contribute to a better understanding of the tolerance mechanisms employed by filamentous fungi and support the optimization of their application in the bioremediation of phenol-contaminated soils and aquatic environments.

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Keywords: filamentous fungi, catechol, biodegradation, superoxide dismutase (SOD), catalase, oxidative stress, bioremediation

Antimicrobial Potential of Filamentous Fungi Isolated from Antarctic Soils on Livingston Island

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Abstract

Microbial resistance represents one of the most pressing challenges in contemporary medicine and biotechnology, driving the ongoing search for novel bioactive compounds with unique antimicrobial properties. The aim of the present study was to determine the biological potential of newly isolated filamentous fungi from Antarctica for the production of antimicrobial metabolites. A total of 71 filamentous fungal strains were isolated from 11 soil samples collected at selected sites on Livingston Island, Antarctica. The temperature range for growth and the optimal growth temperature of the isolates were determined. Most of the isolates were classified as mesophilic fungi. The ability of the isolates to produce compounds with antibacterial and antifungal activity was investigated. Antibacterial activity was evaluated against five bacterial species: two Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and three Gram-negative bacteria (*Pseudomonas aeruginosa, Klebsiella pneumoniae, and Escherichia coli*). Antifungal activity was tested against one yeast species (*Candida albicans*) and three filamentous fungi (*Aspergillus niger, Aspergillus fumigatus*, and *Penicillium griseofulvum*). Of the 71 isolates, 25 exhibited antibacterial activity: four were active only against Gram-positive bacteria, eight only against Gram-negative bacteria, and eleven against both. Isolates III 45N and III 49N showed antibacterial activity against all bacterial species included in the study. Five isolates tested positive for β-lactam antibiotic production. Compared with antibacterial activity, antifungal activity was less frequently observed, with only 16 isolates exhibiting inhibitory effects against the tested fungal species. Among the newly isolated strains displaying activity against both bacteria and fungi, five were selected for further investigation due to their broad-spectrum antimicrobial potential.

This study was funded by the Bulgarian Ministry of Education and Science through the National Centre for Polar Studies and Sofia University “St. Kliment Ohridski” in the framework of the National Program for Polar Studies 2022–2025, project 70-25-99/23.06.2025 witch we would like to express our heartfelt thanks.

Keywords: filamentous fungi, Antartic, antimicrobial compounds

Cellulose-degrading fungi from native environmental sources with potential to break down waste during manned spaceflights

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Abstract

The accumulation of cellulose-rich waste from agricultural, industrial, and domestic activities presents a significant environmental challenge. Furthermore, effective waste management is critical for long-duration space missions, where waste must be processed and recycled in situ. Fungi are the primary agents of cellulose degradation in nature, and their cellulolytic capabilities are the subject of intensive research. This study aimed to isolate, identify, and evaluate fungal strains with high cellulolytic potential from diverse natural habitats, including decomposing wood, bat guano, and forest soil. Fungal strains were isolated using the dilution plate method on Cooke Rose Bengal agar, with incubation at 4°C, 10°C, and 25°C for up to three weeks. Pure cultures were obtained by subculturing hyphal tips, colonies, or spores onto Potato Dextrose Agar (PDA), Sabouraud Agar (SA), and Beer Agar (BA). Pure isolates were maintained on BA slants at 4°C. Taxonomic identification was performed using a combination of morphological analysis and molecular techniques. The ITS region and SSU ribosomal RNA gene sequences were deposited in the NCBI GenBank database. The cellulase activity was measured by growing the fungi on two different types of agar: carboxymethyl cellulose (CMC) and microcrystalline cellulose (MCC). The staining of the agar with Congo red was then used to visualize the enzyme activity, and the clear hydrolysis zone diameter was calculated. Nineteen fungal strains were isolated from decomposing wood and identified morphologically as members of the genera *Trichoderma, Penicillium, Aspergillus*, and *Cladosporium*. Molecular analysis confirmed the identity of four specific strains: *Trichoderma atroviride, Talaromyces pinophilus*, and *Aspergillus tamarii.* From the prehistoric paintings in Magura Cave, Bulgaria, which contain bat guano, 14 pure cultures were obtained. These were identified via molecular methods as representatives of the phyla *Zygomycota* (genera *Mucor* and *Mortierella*) and *Ascomycota* (genera *Aspergillus, Penicillium, Cladosporium, Pseudogymnoascus*, and *Stachybotrys*). Additionally, fungal strains isolated from forest soil were identified as *Daedalea quercina, Lenzites warnieri, Fistulina hepatica, Schizophyllum commune, Trametes trogii,* and *Trametes hirsuta.* Strains isolated from forest soil demonstrated the highest extracellular cellulase

production, followed by those from decomposing wood. *Talaromyces pinophilus* (from wood), as well as *Schizophyllum commune* and *Trametes hirsute* (from forest soil), were identified as the most promising candidates for future biotechnological applications and will be the focus of subsequent investigations. The ability of cellulolytic enzyme production could be useful for the development of technology concerning waste degradation in space flight conditions.

The authors are grateful to the Bulgarian National Science Fund at the Ministry of Education and Science, Republic of Bulgaria, grant number KP-06-H71/8.

Keywords: fungi, cellulose waste, isolation, identification, cellulolytic potential

Comparison of the Cellulase Production Capacity of *Cladosporium* sp. Isolated from a Historical Manuscript under Different Culture Media, pH, and Temperature Conditions

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Abstract

Historical manuscripts and other paper-based cultural heritage materials are vulnerable to biological, chemical, and physical deterioration. Among the biological agents, cellulolytic fungi play a major role by degrading cellulose, the principal structural component of paper. This study aimed to evaluate the cellulase production capacity of a cellulolytic *Cladosporium* sp. isolated from a historical manuscript preserved in the Süleymaniye Manuscript Library (Istanbul, Türkiye) under different culture media, pH, and temperature conditions and to determine the optimum conditions for enzyme production. A fungal isolate previously identified as *Cladosporium* sp. by molecular methods was used. The isolate, preserved at -86 °C, was revived on potato dextrose agar and incubated at 25 °C. Cellulase production was evaluated on four solid media containing carboxymethyl cellulose under four pH levels (5-8) and five incubation temperatures (18-27 °C). Following incubation, Gram's iodine solution was applied to visualize cellulase activity by the formation of clear hydrolysis zones around fungal colonies. Colony and hydrolysis zone diameters were measured, and the enzyme index was calculated as the ratio of hydrolysis zone diameter to colony diameter. The highest cellulase index (2.5) was obtained on El Bergadi medium at pH 7 after 7 days of incubation at 18°C. The findings demonstrate that culture medium composition, pH, and temperature significantly influence cellulase production by *Cladosporium* sp. Furthermore, the ability of the isolate to produce cellulase efficiently at relatively low temperatures suggests that cellulosic materials may remain susceptible to fungal biodeterioration even under low-temperature storage conditions commonly used in archives and libraries. These findings provide valuable insights into fungal-mediated biodeterioration and support the development of effective conservation strategies for paper-based cultural heritage.

Keywords: *Cladosporium* sp., cellulase, historical manuscript

An Evaluation of Biocomposite Insulation Panels Produced From Mushroom Mycel on Existing Patents and Industrial Applicability Potential

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Abstract

Construction sector; It poses significant environmental problems in terms of sustainability due to high energy consumption, use of fossil-based materials and waste generation. In this context, biocomposites developed using fungal mycelium attract attention as an alternative to traditional petroleum-based insulation materials thanks to their renewable and biodegradable properties. Mycelium; Thanks to the hypha network that brings together lignocellulosic agricultural waste as a natural binder, it allows composite materials to be obtained with low-energy production processes. According to literature data, mycelium biocomposites stand out with their low thermal conductivity, high acoustic insulation capacity, inherent fire retardant properties, and carbon-negative production potential. However, high water absorption behavior and limited mechanical strength have been identified as the main technical constraints to the widespread use of the material. It is also seen that these problems can be significantly improved with natural coatings, biopolymer additives, hybrid composite systems and appropriate cork species selection. In this study, the technical and industrial applicability of mycelium-based biocomposite insulation panels in the construction sector; It was evaluated based on national and international literature and patents published between 2010-2025. Patent analyses show that foreign studies focus on basic production processes and biofabrication methods; domestic patents show that it develops application-oriented engineering solutions such as water resistance, mechanical strength and integration into building systems. In conclusion, the study reveals that mycelium-based biocomposite insulation panels have high application potential in the sustainable building sector; By evaluating the literature and patent data together, it presents current technological trends, research gaps and development areas that can guide future R&D studies from a holistic perspective.

Keywords: Mycelium biocomposite, fungal biotechnology, sustainable building materials, patent analysis

Molecular Identification and Thermal Resistance of Heat-Resistant Molds Isolated from Foods: Influence of pH on Ascospore Heat Resistance

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Abstract

Heat-resistant molds (HRMs) are among the most significant spoilage microorganisms in thermally processed foods because their ascospores can survive conventional pasteurization and subsequently germinate during storage, resulting in economic losses and potential food safety concerns. This study investigated the diversity of HRMs isolated from different food products and assessed the effects of pH and temperature on the thermal resistance of their ascospores. After phenotypical identification, selected isolates were identified by MALDI-TOF MS and PCR-based molecular analyses as *Paecilomyces niveus, Aspergillus hiratsukae, Monascus ruber, Aspergillus terreus*, and *Aspergillus niger*. Thermal resistance experiments were conducted on two isolates (*P. niveus* and *A. hiratsukae*) at pH 3.5 and 7.0 under thermal treatments at 85, 90, and 93 °C. Decimal reduction times (D-values) were calculated to characterize ascospore heat resistance. At pH 3.5, *A. hiratsukae* exhibited greater heat resistance than *P. niveus*, with D-values of 8.34 min (D_{85}), 2.06 min (D_{90}), and 1.73 min (D_{93}). In contrast, at pH 7.0, P. niveus showed superior thermal resistance, particularly at 90 and 93 °C, with D-values of 1.72 and 0.87 min, respectively. For both species, D-values decreased consistently with increasing treatment temperature, confirming the expected enhancement of thermal inactivation at higher temperatures. The observed thermal resistance profiles were consistent with previously reported data, confirming that ascospore heat resistance is strongly influenced by both fungal species and environmental pH. The results further demonstrate substantial interspecies variability in HRMs, indicating that responses to thermal processing cannot be generalized across species. These findings provide new insights into the combined effects of pH and temperature on HRM survival and highlight the importance of designing product-specific thermal processes based on the target spoilage species. Such an approach may improve process validation, minimize the risk of spoilage, and enhance the microbiological safety and shelf life of thermally processed foods.

Keywords: Heat-resistant molds, ascospore heat resistance, thermal inactivation, foodborne fungi.

Investigation of Mycotoxin Presence in Some Unpackaged Spices Obtained From Herbalists in Edirne Province Using The LC-MS/MS Method

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Abstract

Spices are among the food matrices that are highly susceptible to the growth of mycotoxin-producing molds under unsuitable production, drying, and storage conditions. Mycotoxins are important food contaminants that pose significant risks to human and animal health due to their carcinogenic, mutagenic, teratogenic, hepatotoxic, nephrotoxic, and immunosuppressive effects. The aim of this study was to investigate the presence of major mycotoxins in various spice samples obtained from herbalists operating in Edirne Province using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and to evaluate the findings according to the Turkish Food Codex Contaminants Regulation. A total of 48 spice samples, including black cumin (Nigella sativa), black pepper, cumin, red pepper flakes, ground red pepper, cinnamon, ground ginger, and allspice, were collected from six different herbalists (A1–A6) in Edirne Province. The samples were analyzed for the presence of deoxynivalenol (DON), aflatoxins (AFB1, AFB2, AFG1, and AFG2), fumonisins (FB1 and FB2), ochratoxin A (OTA), and zearalenone (ZON). Mycotoxin analyses were performed using the LC-MS/MS method, which enables simultaneous determination of multiple mycotoxins. According to the results, deoxynivalenol was not detected in any of the 48 spice samples. Aflatoxin B1 was detected in 11 (22.9%), aflatoxin B2 in 7 (14.6%), aflatoxin G1 in 5 (10.4%), and aflatoxin G2 in 7 (14.6%) samples. Fumonisin B1 was detected in all samples (100%), whereas fumonisin B2 was detected in 46 (95.8%) samples. Zearalenone was detected in 8 (16.7%) samples, while ochratoxin A was detected in 2 (4.2%) samples. According to the Turkish Food Codex Contaminants Regulation, the red pepper flakes sample obtained from herbalist A3 exceeded the legal limit for aflatoxin B1, whereas the ground red pepper sample obtained from herbalist A5 exceeded the legal limits for aflatoxin B1, total aflatoxins, and ochratoxin A. Since no maximum limits have been established for fumonisins, zearalenone, and deoxynivalenol in spices under the Turkish Food Codex Contaminants Regulation, only their occurrence was reported, and no regulatory compliance assessment could be performed. In conclusion, various mycotoxins were detected in some spice samples sold by herbalists in Edirne Province, and several samples exceeded the legal limits specified in the Turkish Food Codex Contaminants Regulation. Among the spices examined, cinnamon, ground ginger, and red pepper flakes were identified as the most prominent spices in terms of multiple mycotoxin contamination and high mycotoxin levels. These findings demonstrate the critical importance of maintaining appropriate hygiene and storage conditions throughout the production-to-consumption chain and implementing regular mycotoxin monitoring programs to ensure consumer safety.

Keywords: Spice, mycotoxin, aflatoxin, ochratoxin A, fumonisin, zearalenone, LC-MS/MS

Comparative Evaluation of Laccase Activities in Different Macrofungi Collected from the Istranca Forests and Their Application in Amperometric Biosensor Design

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Abstract

Macrofungi are prominent natural sources of ligninolytic enzymes that act as effective biocatalysts in the oxidative transformation of lignocellulosic biomass. Among these enzymes, laccases (EC 1.10.3.2) have attracted particular attention as versatile biocatalysts with broad biotechnological applicability. Laccases belong to the multicopper oxidase superfamily and catalyse the oxidation of a broad range of phenolic and aromatic compounds while reducing molecular oxygen to water. Owing to their favourable redox properties, broad substrate specificity, and ability to function under mild reaction conditions, macrofungal laccases are suitable for diverse analytical and biotechnological applications. In particular, laccase-based amperometric biosensors have emerged as analytical platforms for the sensitive detection of phenolic compounds. In this study, crude enzyme extracts were prepared from five macrofungal specimens—*Russula* sp. 1, *Russula* sp. 2, *Tremella mesenterica*, *Agrocybe* sp., and *Amanita rubescens*—collected from the Istranca Forests in Kırklareli, Türkiye, to investigate their potential as sources of laccase. Crude enzyme extracts were prepared from the macrofungal tissues using 0.15 M NaCl followed by filtration and centrifugation. Specific laccase activities were calculated from volumetric activities determined using ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] and protein concentrations measured by the Bradford method. The specific activities of the crude extracts from *Russula* sp. 1, *Russula* sp. 2, *T. mesenterica*, *A. rubescens*, and *Agrocybe* sp., were determined as 0.10455, 0.09379, 0.03356, 0.02951, 0.01167 U mg^{-1}, respectively. Based on these results, Russula sp. 1, which exhibited the highest specific laccase activity, was selected for biosensor construction. For biosensor fabrication, a separate tissue homogenate of Russula sp. 1 was prepared in 0.15 M NaCl and used as the biological recognition element. The tissue homogenate was mixed with graphite powder and mineral oil to prepare the tissue-modified carbon paste, which was then packed into the electrode body. The electrode surface was subsequently coated with a gelatine layer and cross-linked with glutaraldehyde. The resulting working electrode was incorporated into a three-electrode system for the amperometric detection of gallic acid as a model phenolic compound. Under the selected operating conditions of 100 mM sodium-citrate buffer at pH 5.5, 35 °C, and an applied potential of +0.7 V (versus Ag/AgCl), the biosensor exhibited a linear amperometric response to gallic acid over the concentration range of 2.5–100 µM, with an R^2 of 0.9996. In conclusion, this study provides a preliminary assessment of the investigated macrofungal specimens as potential sources of laccase and

reveals variation in the specific laccase activities of their crude extracts. Furthermore, the incorporation of a tissue homogenate of *Russula* sp. 1 into a carbon paste electrode yielded a linear response to gallic acid, demonstrating its potential as a biological recognition element for amperometric biosensor development. These findings provide a foundation for further research into macrofungal enzyme-based biosensors, while also paving the way for a more comprehensive evaluation of the investigated macrofungal specimens as potential sources of laccase through molecular identification and further purification and characterisation studies of their laccases.

Keywords: Laccase, amperometric biosensor, carbon paste electrode, *Russula* sp., gallic acid

Optimizing Substrate Composition and Mineral Additives for *Ganoderma lucidum*-Based Biocomposite Production

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Abstract

Ganoderma lucidum is a white-rot fungus with antioxidant, antimicrobial, and anti-inflammatory properties, as well as the ability to degrade lignocellulosic materials. Its ability to form a strong mycelial network gives it significant potential for producing environmentally friendly and sustainable biomaterials from organic waste. This study investigated mycelial growth of *Ganoderma lucidum* across various substrate formulations to fabricate mycelium-based biocomposites. To evaluate their effects on mycelial growth, different ratios of wheat grain to hemp sawdust (10:90, 25:75, and 50:50) were tested as primary substrates, supplemented with calcium carbonate ($CaCO_3$) and calcium sulfate ($CaSO_4$) at varying concentrations (0%, 1%, and 2%). A total of 27 experimental groups were analyzed in triplicate. *G. lucidum* pileus explants were surface-sterilized using a 1% sodium hypochlorite solution for 20 minutes, inoculated onto potato dextrose agar (PDA), and subsequent mycelial cultures were transferred to sterilized substrates for incubation. Colonized substrate mixtures were supplemented with a 10% wheat flour suspension, combined with additional hemp sawdust, packed into molds, and thermally dried upon full mycelial growth to obtain biocomposite samples. Evaluation revealed that mycelial colonization was significantly influenced by substrate composition and mineral additive combinations. The most robust mycelial growth occurred in substrates containing 50% wheat grain and 50% hemp sawdust, supplemented with dual mineral combinations (1% $CaCO_3$ + 1% $CaSO_4$, 1% $CaCO_3$ + 2% $CaSO_4$, and 2% $CaCO_3$ + 1% $CaSO_4$). Conversely, the lowest growth was observed in the 10:90 wheat-to-hemp formulation. Furthermore, combined $CaCO_3$ and $CaSO_4$ usage resulted in higher colonization rates compared to control groups or single-additive treatments, demonstrating a clear synergistic effect. Considering growth efficiency and production economy, the 50:50 wheat grain to hemp sawdust formulation was selected for biocomposite fabrication. These results show that tuning the substrate composition is key to accelerating mycelial growth during biocomposite fabrication. Overall, this work highlights a practical approach to optimizing mycelium-based material production, offering viable options for packaging, construction, and interior design.

Keywords: *Ganoderma lucidum*, biocomposite, sustainable biomaterials, mycelium-based composite, substrat optimization, lignocellulosic biomass, hemp hurd, *Cannabis sativa*

Mold Fermentation in Professional Kitchens and Emerging Gastronomic Trends

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Abstract

Contemporary culinary practice has increasingly engaged with the basic sciences, moving beyond conventional recipe-based approaches toward controlled and science-driven food processes. One of the most tangible examples of this development is the growing use of controlled mold fermentation in professional kitchens. Food-grade and well-characterized starter cultures of *Aspergillus oryzae, Penicillium nalgiovense*, and selected *Rhizopus spp.* have been shown to provide chefs with considerable versatility in enhancing umami perception, developing distinctive aroma profiles, modifying food textures, and valorizing underutilized food resources. However, scientific literature addressing the technical understanding of chefs, their awareness of food safety risks, including mycotoxin-related hazards, and their motivations for adopting these processes remains limited. The present review examines the emerging role of controlled mold fermentation in professional culinary practice from a chef-oriented perspective, with particular emphasis on the balance between flavor innovation, culinary functionality and food safety. The extant literature was consulted to ascertain the effectiveness of validated starter cultures, strain-specific safety considerations, mycotoxin risk management, standardized fermentation conditions, and interdisciplinary collaboration. The text pays particular attention to the distinction between controlled fermentation with food-grade starter cultures and uncontrolled growth of environmental molds, some of which may be toxigenic. The extant literature suggests that controlled mold fermentation has the potential to broaden the range of conventional flavors and textures, whilst concurrently contributing to sustainable culinary practices. This is achieved through the valorization of underutilized plant-based substrates, the development of novel plant-based protein foods, and the diversification of resource-efficient menus. The successful integration of safe, science-based mold fermentation techniques into professional kitchens therefore requires a multidisciplinary framework that brings mycology, food engineering, food safety, and gastronomy together through a shared scientific language.

Keywords: Mold fermentation, culinary innovation, chef perspective, food safety, mycotoxin risk

Evaluation of Chitosan Production Potential of Soil-Borne Zygomycetes Under Different Cultivation Conditions

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Abstract

Chitosan, a versatile biopolymer with broad industrial, agricultural, and biomedical applications, is conventionally extracted from crustacean shells. Fungal mycelia, however, provide an environmentally friendly, season-independent, and potentially more consistent alternative source. This study investigates the isolation, genus-level taxonomic identification, and chitosan production potential of soil-borne Zygomycetes under varying cultivation conditions and culture media. Soil samples collected from diverse agricultural environments were subjected to selective isolation procedures to obtain distinct fungal strains. Taxonomic identification of isolates belonging to the genera Absidia, Mucor, and Rhizopus was performed using traditional morphological characterization. To optimize chitosan production, the fungal strains were cultivated in two liquid media—potato dextrose broth and yeast extract peptone dextrose broth—at three initial pH levels (4.0, 5.5, and 7.0) and three incubation periods (3, 5, and 7 days) at 25 °C. Fungal biomass was subsequently harvested, and chitosan was extracted via an alkali–acid treatment process. Production efficiency was evaluated based on chitosan yield relative to dry fungal biomass. The most favorable condition identified was cultivation in potato dextrose broth at pH 5.5 for 7 days, yielding 206.070, 384.328, and 236.486 mg/g of dry biomass from Mucor, Rhizopus, and Absidia, respectively. Among the evaluated genera, Rhizopus exhibited the highest chitosan yield. Representative isolates from each genus are currently undergoing further analytical characterization to assess their chitosan production profiles and polymer properties. These preliminary findings highlight the potential of soil-borne Zygomycetes as alternative sources of fungal chitosan and provide a foundation for further optimization of cultivation conditions toward sustainable chitosan production.

Keywords: Zygomycetes, fungal chitosan, soil fungi, fermentation

Marine Biomass Valorization through Fungal Fermentation: A Biostimulatory Approach

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Abstract

The seasonal accumulation of marine biomass in coastal areas and lagoons, also observed along the coasts of İzmir, represents a regional environmental challenge while simultaneously offering an underutilized biological resource with biotechnological potential. In this context, the effects of *Ulva* sp. and *Posidonia oceanica* biomasses on the growth and metabolic responses of Basidiomycota fungi were investigated. Daedaleopsis confragosa and two different *Ganoderma* isolates were cultivated in control, *Ulva*-supplemented, and *P. oceanica*-supplemented media, and their responses were evaluated based on dry biomass production and laccase activity. *Ulva* supplementation increased biomass production in all isolates, with the most pronounced responses observed in *Ganoderma* sp. 5.1 and *D. confragosa*. Biomass production increased by approximately 2.5-fold, while maximum laccase activity in *D. confragosa* increased by 3.21-fold compared with the corresponding control. In contrast, the effects of *P. oceanica* supplementation showed a more variable profile among the isolates. These findings suggest that *Ulva* biomass may function not only as a nutritional supplement but also as a potential biostimulant capable of modulating fungal growth and enzymatic responses. Accordingly, the *D. confragosa* + *Ulva* condition was selected, together with its corresponding control, for further evaluation of biological activity and nutraceutical potential. Overall, this study aims to contribute to the development of a sustainable, circular, and applicable biotechnological approach for converting environmentally challenging marine biomass into value-added product candidates through fungal biotransformation.

Keywords: Marine biomass, fungal fermentation, laccase, biomass valorization

Macrofungal Diversity, Ecology and Biogeography

Cellular response of halotolerant fungi from the Bulgarian Black Sea region against salt stress

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Abstract

Extreme habitats and the organisms inhabiting them have provoked serious scientific interest in recent years. Filamentous fungi exposed to high salt concentrations (NaCl) experience strong osmotic and ionic stress, which leads to excessive ROS production and subsequent oxidative stress. To adapt and thrive under such harsh conditions, microorganisms have evolved a wide range of adaptive mechanisms. The aim of the present study is to compare the adaptation mechanisms of two different species of filamentous fungi with different degrees of halotolerance to salt stress. The model strains were isolated from soil samples in close proximity to saline sources in the Bulgarian Black Sea region. One was isolated from the Pomorie Lake area and was identified as *Penicillium chrysogenum*, and the second was isolated from Atanasovsko Lake and was identified as *Trichoderma afroharzianum*. The cellular response of both the model strains, cultivated in the presence of different concentrations of sodium chloride against salt stress, was compared. The results obtained show that the strains show different growth ranges. *T. afroharzianum,* which is weakly halotolerant, clearly shows increased sensitivity to high concentrations of sodium chloride. A clear increase in the levels of cellular biomarkers of oxidative stress and a significant stimulation of antioxidant defense were observed. The present study confirms the relationship between salt and oxidative stress and the essential role of antioxidant enzymatic defense in the survival of eukaryotic cells under extreme conditions.

Keywords: halotolerant fungi, salt stress, cellular response

Plant Pathology and Agricultural Mycology

First Occurrence of *Fusarium equiseti* Infecting Cotton (*Gossypium hirsutum* L.) in Azerbaijan

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Abstract

Fusarium wilt is one of the most destructive diseases limiting cotton production worldwide, causing significant losses in both yield and fiber quality. In Azerbaijan, where cotton is an economically important crop, information regarding the diversity of *Fusarium* species associated with wilt disease remains limited. This is largely due to the lack of comprehensive studies identifying the causal agents of the disease, resulting in the application of pesticides that target different pathogens rather than the actual causal organism. Such practices not only contribute to environmental pollution but also promote the development of resistant pathogen populations, leading to substantial economic losses. Therefore, the present study aimed to isolate, identify, and characterize the causal agent of wilt disease from infected upland cotton (*Gossypium hirsutum* L.) plants collected from the Goranboy region of Azerbaijan. Symptomatic stem tissues were surface-sterilized, cultured on potato dextrose agar (PDA), and purified for further analyses. The isolates were characterized based on their morphological and cultural features, followed by genomic DNA extraction, PCR amplification, DNA sequencing, and phylogenetic analyses for molecular identification. To ensure accurate species identification and reliable discrimination among closely related taxa, molecular analyses were performed using 6 different genetic markers and primer pairs: ITS1–ITS4, NL11–CNS1, FEF1–FER1, CLOX1–CLOX2, ACT_512F–ACT_783R and CAL_228–CAL_737R. Based on morphological observations and molecular characterization, the pathogen was identified as *Fusarium equiseti.* The detection of *F. equiseti* in cotton fields suggests that this species may represent an important pathogen affecting cotton production in Azerbaijan and highlights the necessity for accurate diagnosis and continuous monitoring of *Fusarium*-associated diseases. The findings of this study provide baseline data for future epidemiological investigations and contribute to the development of effective disease management strategies for sustainable cotton production.

Keywords: *Fusarium equiseti, Gossypium hirsutum* L., cotton, molecular characterization, Azerbaijan

Evaluation of the Antifungal Activity of Clove, Mustard, and Black Cumin Seeds against Phytopathogenic Fungi

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Abstract

In this study, the in vitro antifungal activities of powdered seeds and commercially available pure oils of clove, mustard and black cumin were investigated against three molecularly identified phytopathogenic fungi (*Fusarium proliferatum*, *Botrytis cinerea* and *Trichothecium roseum*). The antifungal effects of volatile-phase compounds from different amounts and concentrations of seed powders (1, 0.5, 0.1, and 0.05 g) and oils (5 and 1 mL) were evaluated using the double-plate volatile-phase method, without direct contact between the test materials and the fungi. In the volatile-phase experiments, distilled water was added to specified amounts of seed powders, while the pure oils were used directly. Fungal cultures containing no test material were used as controls, and all experiments were conducted in triplicate. Mycelial growth was measured daily for seven days, and mycelial growth inhibition (MGI) percentages were calculated. Additionally, the minimum inhibitory concentrations (MIC) of the pure oils and aqueous extracts prepared from the powdered seeds were determined using the microdilution method. The results revealed that clove and mustard seed applications exhibited particularly high antifungal activity. Clove powder (1 and 0.1 g) and clove oil (5 mL) resulted in 100% inhibition of mycelial growth in all tested fungi. While 1 g of mustard seed powder caused 100% MGI in all tested fungi, 0.05 g also resulted in 100% inhibition against *T. roseum*. Application of 0.1 g mustard seed powder provided complete inhibition of *B. cinerea* throughout the seven-day incubation period. Clove oil at 1 mL caused 100% inhibition of *F. proliferatum* and *T. roseum*, and 70.98% inhibition of *B. cinerea*. Black cumin powder showed comparatively lower antifungal activity. MIC results indicated that mustard seed oil and clove oil were effective at lower concentrations, with the lowest MIC (62.5 µg/mL). In addition, the oils exhibited higher antifungal activity than the aqueous extracts. These findings indicate that volatile compounds derived particularly from clove and mustard have considerable potential for the development of natural antifungal agents.

Keywords: Antifungal activity, volatile compounds, phytopathogenic fungi, mycelial growth inhibition, minimum inhibitory concentration

Fungal Symbioses: Mycorrhizae, Lichens and Associated Interactions

Coordinated Optimization for Sustainable Mediterranean Agroforestry with Truffles and High-Value Species (COSMOS-PRIMA project)

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Abstract

Although agroforestry has recently gained prominence as a system for increasing climate resilience and the sustainability of agricultural systems and also conserving biodiversity, it has a long history in Mediterranean-type ecosystems. Agroforestry systems play a significant role in mitigating, preparing and facilitating adaptation to climate change through ways such as soil improvement and water retention by capturing atmospheric carbon dioxide. The Mediterranean region is one of the hotspots where the effects of climate change are intensely felt. Interest in agroforestry is growing every day due to reasons such as a better understanding of its benefits and climate change scenarios, and the analysis of scenarios that will occur in the Mediterranean region. This interest is reflected in the projects presented in this field, and researchers working on the subject are expected to produce practical and innovative projects. One of these is the PRIMA project titled “Coordinated Optimization for Sustainable Mediterranean Agroforestry with Truffles and High-Value Species (COSMOS)”, which will be carried out between 2025 and 2028. The COSMOS project involves 12 partners from 6 countries: Croatia, France, Italy, Slovenia, Spain, and Türkiye. During the project, the goals are to revitalize agroforestry systems to ensure sustainability and efficiency, focusing on vineyards, medicinal and aromatic plants, fruit trees, and truffles combined with species supporting pollen and honey production; to explore market access and value chains for various agroforestry products; to identify technical, political, and socio-economic

barriers hindering the adoption of sustainable agroforestry practices and to provide policy recommendations to overcome these challenges; and to raise awareness, understanding, and knowledge of agroforestry systems among different stakeholders through gender-sensitive training programs. For these objectives, 12 demo sites and 6 living laboratories have been established or are under construction in the partner countries.

The COSMOS project is part of the PRIMA programme supported by the European Union and funded by national bodies. The Turkish part of this project was supported by the Scientific and Technological Research Council of Türkiye-TÜBİTAK (project number 125N005).

Keywords: Agroforestry, Mediterranean, truffle

Evaluation of the Relationship Between Epiphytic Lichen Diversity and Air Quality: A Comparative Study in Edirne and Çorlu Urban Areas

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Abstract

Air pollution is a major public health problem that threatens human health. According to World Health Organization, approximately 7 million people die prematurely each year due to exposure to air pollution. It has been observed that lichen flora becomes poorer in regions with high air pollution. Lichens are widely used as biological indicators is common in determining air pollution. Unlike devices that measure air pollution, the use of lichens as biological indicators provides long-term information rather than instantaneous data. Within this scope, it was aimed to evaluate the relationship between epiphytic lichen diversity and air quality in two different urban areas. Within the scope of the research, epiphytic lichen samples were collected from ten sampling points determined in Edirne and Çorlu in 2025 and identified at the genus level. Lichen atlases and identification keys were used for identification. Air quality data for urban areas in 2025 was obtained from the National Air Quality Monitoring Network. The data was compared with the identified lichen samples. Additionally, the spatial distribution of epiphytic lichens at the sampling points was evaluated. The results showed that the genera Physcia, Xanthoria, Lecanora, Evernia, and Ramalina were found in in both study areas. Additionally, it was determined that the genera Physconia, Lecidea and Punctelia were only identified in Çorlu. When air quality data was examined, it was observed that NO_2 and SO_2 concentrations were higher in Çorlu, while PM_{10} concentration was higher in Edirne Previous studies have reported that PM_{10} concentrations may be high due to agricultural activities and biomass burning. In addition, a negative correlation between PM_{10} concentration and lichen diversity has also been reported. The findings of this study suggest that epiphytic lichens can be used as bioindicators in the evaluation of air quality and the importance of interpreting different air quality data together.

Keywords: Lichen, air pollution, bioindicator, diversity

Fungal Natural Products and Chemical Ecology

Antitumor, Anriviral and Anti-Inflammatory Potential of Antarctic Fungal Strains

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Abstract

Antarctic fungi, as inhabitants of extreme environments, have evolved the ability to produce metabolites with unique properties. In the present study, two Antarctic fungal strains, *Penicillium olsonii* p14 and *Penicillium griseofulvum* p29, were compared based on their total polyphenol content (TPC), total flavonoid content (TFC), antioxidant activity (DPPH and ABTS), and antiherpetic activity. Our aim was to identify Antarctic fungal strain with promising biotechnological and pharmaceutical potential. Higher amounts of TPC and TFC were estimated in the low molecular fraction (0–10 kDa) of *P. olsonii* p14 whereas in *P. griseofulvum* p29 TPC was predominantly associated with the >10 kDa fraction and TFC levels were low across all fractions. In both strains, ethanol extracts showed higher DPPH and lower ABTS radical scavenging activity. The monitoring of antiherpetic activity against human herpesvirus type 1 (HSV-1, Victoria strain) was done by assessing the cytopathic effect of the virus against MDBK cells. All studied aqueous fractions showed a remarkable effect on extracellular HSV-1 virions, as well as on the stage of their adsorption to host cells. A significant protective effect on healthy cells against subsequent herpes infection was also observed. Different biosynthetic strategies between the two Antarctic strains were demonstrated. The pronounced production of low-molecular-weight polyphenols and ethanol-soluble flavonoids identify the Antarctic strain *P. olsonii* p14 as a proper candidate for further research as a source of natural bioactive compounds with promising applications in biotechnology and pharmaceutical development.

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Keywords: Antarctica, fungi, metabolites, aqueous extract, ethanol extract

ONLINE PRESENTATIONS

Medical and Veterinary Mycology

Epidemiology of dermatophyte infections in Azerbaijan: Species distribution, gender analysis and clinical correlation A Retrospective microbiological assessment

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Abstract

To determine the species-level distribution of dermatophytes causing superficial fungal infections in Azerbaijan, to evaluate their relationship with patient sex, and to characterise regional epidemiological features; and to compare the findings with global literature in order to contribute to the optimisation of empirical antifungal treatment protocols. Mycological culture results obtained from specimens collected from patients presenting to a microbiology laboratory in Azerbaijan Reference Clinical laboratory center (Narimanov branch) were retrospectively analysed. A total of 80 positive dermatophyte cultures were examined. Male (n=51) and female (n=29) patients were compared. Species identification was performed on Sabouraud Dextrose Agar using macro- and micromorphological criteria. Five distinct dermatophyte species/groups were identified. The most frequently isolated organism was *Trichophyton mentagrophytes* (n=34; 42.5%), followed by *Trichophyton* spp. (n=22; 27.5%), *T. rubrum* (n=21; 26.3%), *M. canis* (n=2; 2.5%) and *T. tonsurans* (n=1; 1.3%). The genus *Trichophyton* was predominant, accounting for 96.3% of all isolates. The overall Male/Female (M/F) ratio was 1.76:1, with male predominance observed across all species groups. The dermatophyte epidemiology of Azerbaijan differs markedly from the *T. rubrum*-dominant profile of Western Europe. The predominance of the zoophilic *T. mentagrophytes* is consistent with occupational exposure related to agriculture and animal husbandry. These findings are of considerable importance for the development of region-specific antifungal protocols and for structuring preventive medicine strategies targeting the male population.

Keywords: dermatomycosis, *Trichophyton mentagrophytes*, dermatophyte epidemiology, Azerbaijan mycology, antifungal therapy, gender analysis, zoophilic dermatophyte

From Sputum Culture to Diagnosis: Multidisciplinary Recognition of Allergic Bronchopulmonary Aspergillosis

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Abstract

Allergic bronchopulmonary aspergillosis is an immune-mediated pulmonary disorder caused by hypersensitivity to *Aspergillus* species. Its clinical and radiological findings may mimic recurrent respiratory infections, leading to delayed diagnosis and repeated courses of empirical antibiotics. A 46-year-old male construction worker presented with a dry cough, dyspnea, episodes of breathlessness and choking sensation, and fatigue that had persisted for several years. He had been evaluated repeatedly by pulmonology clinics and had received multiple courses of empirical antibiotic therapy without sustained clinical improvement. He was also investigated for pulmonary tuberculosis; the purified protein derivative skin test and sputum culture for *Mycobacterium tuberculosis* were negative. Sputum fungal culture yielded *Aspergillus fumigatus*. Following this microbiological finding, an infectious diseases specialist reviewed the patient's clinical history and requested reassessment of the thoracic computed tomography by a radiologist. Reassessment demonstrated varicose bronchiectasis in segments 2 and 3 of the right lung and segment 3 of the left lung, as well as cylindrical bronchiectasis in segment 10 of the left lung and segments 8 and 9 of the right lung. Mucoid impaction producing a characteristic finger-in-glove appearance was also identified. Based on the microbiological and radiological findings, measurement of total serum immunoglobulin E was requested, and the level was found to be markedly elevated at 2,893 IU/mL. Further evaluation was coordinated with pulmonology. Peripheral eosinophil count was unavailable because the complete blood count had been performed using a three-part differential analyzer. Based on the chronic respiratory symptoms, markedly elevated total immunoglobulin E, isolation of *A. fumigatus*, bronchiectatic changes, and mucus plugging, allergic bronchopulmonary aspergillosis was diagnosed. After normal liver function tests were confirmed, oral prednisolone was initiated at 1 mg/kg/day for two weeks, followed by a dose reduction of 10 mg every two weeks over five months. Itraconazole was administered at a loading dose of 800 mg on the first day, followed by 200 mg twice daily. Following treatment, the patient's symptoms regressed significantly. Isolation of *A. fumigatus* from respiratory specimens should not automatically be dismissed as colonization in patients with chronic respiratory symptoms. Correlation of microbiological findings with radiological and immunological features, supported by multidisciplinary collaboration, may enable timely recognition of allergic bronchopulmonary aspergillosis and prevent unnecessary antibiotic exposure and progressive lung damage.

Keywords: allergic bronchopulmonary aspergillosis, *Aspergillus fumigatus*, sputum culture, bronchiectasis, multidisciplinary diagnosis

Diagnostic Challenges in Contemporary Medical Mycology: An Integrated Approach to Invasive Fungal Infections

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Abstract

Invasive fungal infections are increasingly important clinical and epidemiological problems because they are associated with high morbidity, mortality, diagnostic uncertainty, and expanding antifungal resistance. They occur most often in patients with hematological malignancies, prolonged neutropenia, solid-organ or hematopoietic stem-cell transplantation, intensive-care treatment, central venous catheters, broad-spectrum antibacterial therapy, parenteral nutrition, or long-term immunosuppression. *Candida, Aspergillus, Cryptococcus, Pneumocystis jirovecii*, and Mucorales remain the principal pathogens, while *Candida auris*, fluconazole-resistant *Candida parapsilosis*, and azole-resistant *Aspergillus fumigatus* illustrate the growing resistance problem. Early diagnosis remains difficult because clinical and radiological findings are frequently nonspecific. Fever, cough, dyspnea, and pulmonary infiltrates may also occur in bacterial and viral infections, complicating differential diagnosis. Microscopy, histopathology, and culture continue to provide fundamental information. Microscopy can offer rapid preliminary evidence of fungal elements, histopathology can demonstrate tissue invasion, and culture permits isolation, species identification, and antifungal susceptibility testing. Their value is nevertheless limited by low sensitivity, delayed results, dependence on experienced personnel, and the need for invasive sampling in some patients. Moreover, detection of *Candida* or *Aspergillus* in nonsterile specimens may represent colonization rather than invasive disease. Serological biomarkers have become important complementary tools. Galactomannan supports the diagnosis of invasive aspergillosis, particularly in high-risk neutropenic patients, whereas 1,3-β-D-glucan functions as a broad fungal marker and may assist in excluding invasive fungal infection in an appropriate clinical context. Cryptococcal antigen testing, especially in lateral-flow format, provides rapid and reliable detection in serum and cerebrospinal fluid. Combined assessment of mannan antigen and anti-mannan antibodies may strengthen the diagnosis of invasive candidiasis. However, biomarker performance is affected by immune status, specimen type, antifungal exposure, cross-reactivity, colonization, and false-positive reactions. Results must therefore be interpreted together with clinical, radiological, and microbiological findings. Molecular methods, including conventional PCR, real-time PCR, multiplex PCR, panfungal PCR, and T2*Candida*, allow rapid detection of fungal nucleic acids directly in clinical specimens. *Aspergillus* PCR may be applied to bronchoalveolar lavage, blood, serum, plasma, or tissue and can complement galactomannan and imaging. T2*Candida* detects selected *Candida* species directly from blood without prior culture. Molecular assays may also identify resistance-associated markers, including cyp51A alterations in *Aspergillus fumigatus*. Important limitations remain, including

incomplete standardization of specimen selection, DNA extraction, target genes, primer design, amplification platforms, and positivity thresholds. Contamination, cost, limited availability, and persistent DNA positivity after initiation of treatment can further complicate interpretation. Metagenomic next-generation sequencing, microbial cell-free DNA analysis, biosensors, microfluidic platforms, and artificial-intelligence-based algorithms may expand diagnostic possibilities, particularly in culture-negative, atypical, rare, or deep-seated infections. Metagenomic approaches can detect a broad range of microorganisms without restricting analysis to a predefined target and may support outbreak investigation and resistance surveillance. Their routine use is currently limited by cost, specialized infrastructure, bioinformatics requirements, background contamination, human DNA predominance, and the need for clinical validation. Routine application of diagnostic panels in patients with low pre-test probability may generate false-positive findings, unnecessary antifungal exposure, and avoidable costs. Centralizing advanced molecular and sequencing assays in regional or national reference laboratories can improve quality, accessibility, and interpretive consistency. The most appropriate diagnostic model is an integrated, risk-based, and resource-adapted strategy. Initial assessment should combine host risk factors, clinical and radiological findings, microscopy, histopathology, culture, and accessible biomarkers. High-risk, culture-negative, or unresolved cases should progress to PCR-based methods, multiplex platforms, T2*Candida*, or referral-laboratory testing, while metagenomic methods should be reserved for selected complex cases. Appropriate specimen selection is essential: bronchoalveolar lavage is particularly relevant for pulmonary aspergillosis, whereas serum or cerebrospinal fluid is preferred for cryptococcal antigen testing. This stepwise approach can reduce diagnostic delay, support earlier targeted antifungal therapy, limit unnecessary empirical treatment, improve resource use, and contribute to better clinical outcomes.

Keywords: invasive fungal infections, medical mycology, serological diagnostics, molecular diagnostics, galactomannan, β-D-glucan, PCR, *Aspergillus*, *Candida*, antifungal resistance

Comparative Diagnostic Performance of *Aspergillus* Real-Time PCR, Galactomannan Antigen Detection, and Blood Culture in Intensive Care Unit Patients with Probable Invasive Pulmonary Aspergillosis

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Abstract

Invasive pulmonary aspergillosis (IPA) is an increasingly recognized, life-threatening opportunistic fungal infection in critically ill patients. Rapid and precise mycological confirmation in the intensive care unit (ICU) is vital to initiate targeted antifungal therapy and improve clinical outcomes. This study evaluated and compared the diagnostic sensitivity of *Aspergillus* real-time Polymerase Chain Reaction (PCR), Galactomannan (GM) enzyme immunoassay, and conventional hemoculture in critically ill patients classified with probable IPA. A prospective evaluation was conducted on 53 ICU patients presenting with probable IPA classified according to the EORTC/MSGERC consensus criteria. Method-specific positivity thresholds were strictly applied. The GM assay (Platelia *Aspergillus* EIA, Bio-Rad, Marnes-la-Coquette, France) optical density index cutoff was established at 1.0 for serum, plasma, and bronchoalveolar lavage (BAL) fluid. For the *Aspergillus* real-time PCR assay (GeneProof, Brno, Czech Republic), a rigorous multi-specimen positivity algorithm was implemented, requiring either: a minimum of two positive tests from blood compartments (serum, plasma, or whole blood); at least two positive results from BAL fluid; or a combination of at least one positive blood compartment sample alongside one positive BAL fluid sample. Standard automated hemoculture protocols were executed concurrently for all patients. Statistical analysis was performed using McNemar's test and Cochran's Q test to compare the diagnostic sensitivities of the three paired modalities, with 95% confidence intervals (CI) calculated for each parameter. A p-value Biomarker and molecular-based modalities demonstrated significantly higher diagnostic yields in the ICU cohort compared to conventional culture techniques ($p < 0.001$). Out of 53 patients with probable IPA, the *Aspergillus* real-time PCR assay exhibited the highest diagnostic efficacy, testing positive in 52 cases and achieving a clinical sensitivity of 98.11%. The GM antigen detection assay demonstrated strong diagnostic correlation, testing positive in 49 cases, yielding a sensitivity of 92.45%. In contrast, conventional hemoculture demonstrated poor diagnostic utility for this manifestation, yielding positive results in only 11 patients, which translates to a sensitivity of 20.75%. The differences in sensitivity between both PCR and GM compared to hemoculture were highly statistically significant ($p < 0.001$). Molecular and biomarker-driven strategies vastly outperform traditional blood culture methods in diagnosing IPA within the critical care setting. The implementation of *Aspergillus* real-time PCR, using a stringent multi-specimen validation algorithm, delivers optimal

diagnostic sensitivity (98.11%) for cases categorized as probable IPA, closely supported by GM testing (92.45%). Due to the low sensitivity of hemoculture (20.75%), ICU clinical workflows should prioritize parallel PCR and GM testing to ensure rapid, high-sensitivity mycological confirmation and prevent delayed therapeutic intervention.

Keywords: *Aspergillus,* real-time PCR, galactomannan, hemoculture, invasive pulmonary aspergillosis, intensive care unit, EORTC/MSGERC criteria

Species Distribution and Antifungal Susceptibility Profile of *Aspergillus* Bloodstream Isolates from a Critical Care Unit Using EUCAST Methodology

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Abstract

Fungemia caused by *Aspergillus* species is an extremely rare but highly lethal manifestation of invasive aspergillosis in critically ill patients. Due to the emergence of antifungal resistance among filamentous fungi, precise species identification and in vitro susceptibility testing are paramount to guide targeted therapeutic regimens. This study aimed to identify clinical *Aspergillus* bloodstream isolates and evaluate their susceptibility profiles to core mold-active antifungal agents. A retrospective mycological evaluation was performed on 11 *Aspergillus* isolates recovered from positive hemocultures using the BACT/ALERT automated blood culture system. Genotypic-level phenotypic identification was executed using Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry (MALDI-TOF MS, Bruker). Antifungal susceptibility testing (AFST) was conducted via the reference broth microdilution method in strict accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) Definitive Document E.DEF 9.4 guidelines for conidia-forming moulds. Minimum inhibitory concentrations (MICs) were determined for amphotericin B, isavuconazole, posaconazole, and itraconazole, and interpreted using current EUCAST clinical breakpoints. Among the 11 bloodstream isolates identified by MALDI-TOF MS, *Aspergillus fumigatus* was the predominant species (n = 7; 63.6\%), followed by *Aspergillus flavus* (n = 3; 27.3%) and *Aspergillus* terreus (n = 1; 9.1%). In vitro susceptibility testing revealed distinct compound-specific profiles across the cohort. Universal susceptibility was observed for the novel triazole isavuconazole, which demonstrated a 100% susceptibility rate. High susceptibility rates were also sustained for the other triazoles, with posaconazole showing (94.0%) susceptibility and itraconazole showing 92.0% susceptibility. Amphotericin B exhibited an overall susceptibility rate of 91.0%, with reduced susceptibility largely localized to the A. terreus isolate, aligning with its intrinsic polyene resistance profile. *Aspergillus fumigatus* remains the primary etiological agent of *Aspergillus* fungemia in critical care settings, though cryptic non-fumigatus species like A. flavus and A. terreus represent significant clinical threats. The incorporation of high-throughput MALDI-TOF MS enables swift, unambiguous identification. Reference microdilution according to EUCAST E.DEF 9.4 standards indicates that mold-active triazoles—particularly isavuconazole (100%)—retain excellent in vitro efficacy, making them

reliable first-line choices, while polyene utility remains limited by species-specific intrinsic resistance patterns.

Keywords: *Aspergillus* fungemia, MALDI-TOF, EUCAST, isavuconazole, posaconazole, itraconazole

Fungal Systematics, Taxonomy and Nomenclature

Novel *Russula* species in Colombia reveal ecological and evolutive patterns related to host-symbiont interaction with Fagaceae family trees

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Abstract

Russula represents the most diverse and dominant genus among ectomycorrhizal agarics. Despite this diversity, the number of documented *Russula* species in Central and South America remains significantly lower than in the northern hemisphere. In Colombian montane forests, *Russula* forms symbiotic associations with Fagaceae trees, specifically Q*uercus humboldti*i and *Trigonobalanus excelsa.* The distribution and diversification of this host-symbiont complex are shaped by biogeographic separation following the formation of the Isthmus of Panama. To support conservation efforts, preliminary multilocus genetic barcode sequencing was performed on 37 specimens representing different morphotypes, targeting the ITS, 28S, rpb1, rpb2, mcm7, and tef regions. These analyses revealed four lineages, including at least ten novel species: the first lineage, *Russula* subsect. Castanopsidium, comprises two species: one endemic to Colombia and another present in both Colombia and Panama, which may indicate allopatric speciation due to the absence of hosts in the Darien Gap. The second lineage, the recently recognized *Russula paludosa* group, includes two Colombian species and one Panamanian species. The third and fourth lineages, *Russula* sect. Ingratula and *Russula* subsect. Heterophyllidia, are represented by three and four new species, respectively. This ongoing international research advances understanding of Colombia's complex fungal diversity and highlights the urgent need to conserve and study the increasingly threatened monotypic montane forests.

Keywords: Russula, Fagaceae, ectomycorrhiza, Colombia

Dermatophytes in Medical Mycology: Current approaches to clinical and laboratory diagnosis

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Abstract

Dermatophytes are filamentous fungi with a high affinity for keratinized tissues and represent the principal etiological agents of superficial fungal infections (dermatophytosis) affecting the skin, hair and nails. Species belonging to the genera *Trichophyton*, *Microsporum* and *Epidermophyton* are responsible for the majority of dermatophyte infections worldwide. Owing to their high prevalence, recurrent nature and significant impact on patients quality of life, dermatophytosis remain an important global public health concern. In recent years, the increasing number of immunocompromised individuals, the inappropriate use of topical corticosteroids and the emerge of antifungal resistance have created new challenges in the diagnosis and management of those infections. The diagnosis of dermatophytosis should be based on both clinical evaluation and laboratory confirmation. Conventional diagnostic methods, including direct microscopy using potassium hydroxide (KOH), fungal culture and morphological identification, continue to play a fundamental role in routine laboratory practice. In addition, modern molecular techniques such as real-time polymerase chain reaction (Real-Time PCR), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALTI-TOF MS) and internal transcribed spacer (ITS) sequencing provide rapid, accurate and species-level identification of dermatophytes. The recent global emergence of terbinafine – resistant *Trichophyton indotineae* has further highlighted the clinical importance of molecular diagnostics and antifungal susceptibility testing. Effective management of dermatophyte infections requires accurate clinical assessment, appropriate selection of laboratory diagnostic methods and targeted antifungal therapy based on the causative species. The implementation of modern diagnostic approaches contributes to early diagnosis, optimized treatment strategies reduced recurrence rates and improved management of antifungal resistance.

Keywords: Dermatophytes, dermatophytosis, *Trichophyton, Microsporum, Epidermophyton*, laboratory diagnosis, Real-Time PCR, MALDI-TOF MS, antifungal resistance, *Trichophyton indotineae*

Fungal Symbioses: Mycorrhizae, Lichens and Associated Interactions

Use of native soil inoculum for the restoration of root-associated fungal communities in endangered *Juglans neotropica*

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Abstract

Soil fungal communities play a fundamental role in plant survival and development in tropical montane forests; however, restoration and conservation strategies for endangered tree species rarely integrate the soil microbiome. Juglans neotropica, an endangered Andean walnut listed on the International Union for Conservation of Nature Red List, forms symbiotic associations with root-associated fungi that remain unstudied, representing a critical knowledge gap for its ecological restoration. In this study we evaluated the effect of native live soil inoculation from conspecific adult trees on seedling growth, functional traits, and root-associated fungal community diversity of *Juglans neotropica* seedlings from two populations of contrasting geographic origin. We established a nursery inoculation experiment monitoring seedling growth over seven months. Root samples from nursery seedlings grown with and without native soil inoculum, wild seedlings, native bulk soil, and commercial sterile peat were characterized using Illumina sequencing and analyzed for alpha and beta diversity patterns. We found that the seedlings of *Juglans neotropica* grow more when inoculated with live soil from conspecifics; however, this effect was stronger in seedlings from the local population. In addition, we found that seedlings grown in sterile peat show a high abundance of phytopathogens, particularly *Fusarium* and *Dactylonectria.* Our results highlight the importance of including the soil microbiome in propagation processes to increase seedling growth and potentially improve their adaptation to field conditions. Native soil and wild seedlings exhibited the highest fungal diversity and a distinct community composition compared to nursery-grown seedlings. Seedling response to inoculation also depended on the individual inoculum donor tree, suggesting that specific microbial assemblages drive differential plant performance. To our knowledge, this is the first study characterizing the root-associated fungal communities of *J. neotropica* using environmental DNA sequencing. Our findings raise critical concerns about the widespread use of commercial sterile peat in restoration nurseries, as it appears to facilitate phytopathogen proliferation during early seedling stages.

Keywords: Andean walnut, ecological restoration, root-associated fungi, soil microbiome

Fungal Phylogenomics and Molecular Evolution

Genomic consequences of postglacial secondary contact between diverged lineages of *Trichaptum abietinum* in Europe

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Abstract

The effects of secondary contact on the divergence and potential speciation of fungal species are still not understood completely, particularly for basidiomycetes. In this study, we use *Trichaptum abietinum*, a widespread wood-decaying polypore associated with conifers, as our model to test the genomic implications of secondary contact and hybridization. Previous studies have described the existence of divergent genomic lineages of *T. abietinum* in Europe: Boreal and Mediterranean. These lineages diverged in allopatry in glacial refugia and the subsequent secondary contact following postglacial migration has produced the admixed population in Central Europe. Interestingly, this admixed population have a unique pattern of a complete Boreal ancestry located in the second half of Scaffold 5. This suggests a non-random inheritance in the admixed population, which could be the result of selection or genomic incompatibilities. In this work, we focus on Scaffold 5 and how it has been inherited in the admixed population. We performed analyses of fine-scale recombination and coalescence between the parental lineages and compared them to the admixed populations. Additionally, we used the genome sequences from viable spores of the same fruitbody to consider the genomic variation between related individuals obtained from different meiosis in one generation. We found a variable population structure in samples from Czech Republic, showing a Mediterranean ancestry in Scaffold 5, in comparison to what was found in previous studies. The Czech population showed a consistently low recombination rate in Scaffold 5, suggesting selection against introgression in this region. On the other hand, samples from Austria showed a Boreal ancestry in Scaffold 5 with a clear peak of recombination, suggesting high levels of introgression. The results show more complex evolutionary story occurring in on the admixed population of Central Europe and its study important for our further understanding on the hybridization and selection shaping the populations of *T. abietinum*.

Keywords: Recombination landscape, introgression, demography, *Trichaptum abietinum*

Two New Records of *Helvella semiobruta* Donadini & Berthet and *Helvella varia* X.C. Wang & W.Y. Zhuang from Çanakkale, Türkiye

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Abstract

Helvella L. species constitute a taxonomically challenging group within the order Pezizales due to the high variability of apothecial morphology, the occurrence of similar macroscopic characters across different taxa, and the frequent resolution of species boundaries through molecular data. In the present study, two *Helvella* specimens collected from the Terzioğlu Campus of Çanakkale Onsekiz Mart University were evaluated based on morphological characteristics and LSU rDNA sequence data. As a result of field and laboratory investigations, preliminary morphological identifications were made considering apothecial shape, hymenial surface color, external surface characteristics, stipe structure, and features of asci, ascospores, and paraphyses. For molecular confirmation, the obtained LSU rDNA sequences were compared with reference sequences available in public databases, and phylogenetic analyses revealed that the specimens clustered with *Helvella semiobruta* and *Helvella varia*, respectively. *H. semiobruta* is a species particularly associated with Mediterranean-type habitats, characterized by greyish-brown to dark apothecia and a distinctly but generally not strongly ribbed stipe. *H. varia*, on the other hand, is a recently described taxon belonging to the *Helvella lacunosa* species complex, whose delimitation is supported by molecular data. Therefore, the identification of both species requires the use of molecular evidence in addition to morphological characters. Based on the combined evaluation of morphological and molecular data, the examined specimens were confirmed as *H. semiobruta* and *H. varia*. This study reports both taxa for the first time from the Turkish mycobiota and suggests that the diversity of *Helvella* in Türkiye may be higher than previously assumed. Furthermore, the findings indicate that campus and semi-natural Mediterranean-type habitats represent noteworthy areas in terms of ascomycete diversity.

Keywords: Ascomycota, *Helvella*, LSU rDNA, new record, Çanakkale, Türkiye

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