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PATHOGENIC POTENTIAL OF RARE OPPORTUNISTIC SKIN MYCOSES AGENTS ISOLATED FROM ANIMALS IN KAZAKHSTAN

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ABSTRACT

In recent decades, the spectrum of pathogens has shifted, with an increasing proportion of opportunistic and rare fungi involved in the development of skin infections in animals. The aim of this study was to investigate the biological properties of rare opportunistic mold fungi isolated from the skin of farm animals in Kazakhstan. 760 biomaterial samples collected from cattle, sheep, and horses in six regions of Northern and Central Kazakhstan were analyzed. Mycological analysis yielded 281 fungal isolates, including rare representatives of the genera *Chaetomium*, *Trichoderma*, and *Trichothecium*. Isolates were identified using culture-morphological and molecular genetic methods. The enzymatic (proteolytic, urease, saccharolytic), keratinophilic, and keratinolytic activities of the strains were studied. It was established that the rare isolates possess pronounced enzymatic potential associated with pathogenic factors. *Chaetomium globosum* exhibited high keratinophilic and keratinolytic activity. *Trichoderma citrinoviride* exhibited high carbohydrate digestion activity and moderate keratinolytic activity. *Trichothecium roseum* exhibited high overall enzymatic activity and the ability to actively destroy hair.

Keywords: *Chaetomium*; *Trichoderma*; *Trichothecium*; cutaneous mycoses; mold fungi; rare pathogens; enzymatic activity.

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1. INTRODUCTION

Cutaneous mycoses are fungal infections that primarily affect the stratum corneum, or outermost layer of the skin, as well as the mucous membranes, nails, and hair. These infections are among the most common diseases occurring worldwide, with an incidence reaching 20-25% [1, 2]. Over the past 20 years, the prevalence of dermatomycoses in both humans and animals has increased significantly [3, 4].

Over the past two decades, there have been increasing reports of changes in the spectrum of classic dermatomycosis pathogens and the emergence of new opportunistic diseases in animals [5, 6, 7, 8]. Opportunistic mycoses are recorded in most warm-blooded and cold-blooded animals, vertebrates and invertebrates, domestic, wild, commercial, and food-producing animal species. The overall prevalence of opportunistic mycoses increased from 19.3% in 1997-2004 to 19.5%. up to 78.5% in the period 2005-2012 [9]. The causes of mycoses caused by opportunistic and pathogenic fungi are considered to be the intensification of production, changes in environmental factors, stress, and the increase in the proportion of animals with weakened immune systems [10, 11].

A wide range of filamentous and yeast fungi can act as causative agents of opportunistic mycoses [12]. In farm and wild animals, well-known aspergillosis, mucormycosis, candidiasis, cryptococcosis, and infections caused by melanized fungi have been described, often occurring in a malignant form and leading to their death [13, 14].

There are regular reports of new previously unknown, rare and non-trivial causative agents of skin mycoses. Among the new opportunistic fungal pathogens, fungi of the genera *Trichoderma*, *Chaetomium*, *Trichothecium* and a number of other ecologically significant keratinophiles are of particular interest. These fungi are widespread in soils, plant debris, litter, organic substrates, and circulate continuously in animal habitats. They are commonly known as saprophytes in soil or environmental plant decomposition agents, however their ability to produce keratinolytic enzymes allows them to break down protective keratin structures. Fungi of the genus *Trichoderma*, *Chaetomium* and *Trichothecium* are described as rare or newly emerging opportunistic pathogens that cause mycoses of the skin, nails and other parts of the body in humans and immunocompromised animals [15-18]. It was reported that out of 82 clinical samples of animals obtained from skin lesions, 13 mold strains (15.8%) were isolated, of which *Chaetomiumiranianum*, *Trichotheciumroseum*, *Lichtheimia* were isolated in isolated cases [3]. A rare case of horse dermatomycosis caused by *Geotrichum* candidum has been described [19]. *Syncephalastrum* spp. [20], *Phomamacrostoma* [21]. The high potential of *Trichothecium* spp. in the production of mycotoxins (such as trichothecenes), which can

cause toxicosis when contaminated food is consumed, but is usually not reported as a causative agent of skin, hair or nail mycoses. Mention is made of fungi *Trichothecium* spp., which are morphologically very similar to *Microsporum nanum* [22].

The main characteristics of microscopic fungi and yeasts that determine their ability to cause disease are several indicators of pathogenicity: adhesion (attachment), enzymatic activity (proteases, phospholipases, lipases, dimorphism), immune protection factors (capsule, melanin, biofilms), toxin formation, thermal tolerance (fungus ability to actively grow at a temperature of 37°C) [23].

New, previously unknown, fungal pathogens pose a particular danger to veterinary mycologists due to a lack of knowledge about the etiological significance of these mycromycetes and the pathogenesis of the corresponding mycosis in animals, the lack of treatment protocols and the prevention of pathology. Knowledge of the pathogenic potential of rare skin mycosis pathogens will make it possible to quickly adjust the treatment regimen, select the necessary antifungal drug with an effective effect on the pathogenic factor [24].

To better understand future epidemiological trends and risk factors for superficial fungal infections, it is necessary to be informed about rare animal skin mycosis pathogens and their biological properties. The aim of this study was to investigate the biological properties of rare opportunistic mold fungi isolated from the skin of farm animals in Kazakhstan.

2. MATERIAL AND METHODS

A total of 760 biomaterial samples were collected from cattle, sheep, and horses in six administrative districts of Northern and Central Kazakhstan, including 198 in Akmola Region, 95 in Pavlodar Region, 115 in Karaganda Region, 130 in North Kazakhstan Region, 118 in Kostanay Region, and 104 in Ulytau Region.

Biomaterial was collected from animals with visible damage to the skin and its derivatives. Hair was plucked with tweezers, and scale scrapings were taken with a blunt scalpel. [25] Accompanying documents indicating the species, age, and type of affected animal, as well as the collection date, were included with the biomaterial samples.

The biomaterial included the following:

− epidermal flakes scraped with a scalpel from the periphery of fungal lesions;

− epidermis and scabs from blisters or vesicles, swabs from under scabs, and deep swabs from affected areas;

− hairs with their bulbs from the center of the lesion, collected with tweezers;

− pus-containing swabs collected with a dry, sterile cotton swab.

Mycological diagnostics involved the detection of fungal fragments directly in the material collected from the animals and the complete identification of the etiologic factor. Sabouraud's medium was used for the initial isolation of pathogens. The biomaterial was pre-soaked in 70% ethyl alcohol and left for 5 minutes to remove bacterial contamination [26]. To obtain a pure culture, isolates were incubated in an incubator at 28°C for 5 days. Detailed procedures depended on the location of the skin lesions, the type of material being examined, and the suspected etiologic factor.

The presence or absence of fluorescence in the biomaterial from the lesions was determined under a Wood's lamp.

Some of the collected diagnostic material was used to prepare slides for direct microscopic examination. The material was treated with a 10-20% KOH solution supplemented with 40% DMSO (dimethyl sulfoxide) and examined under a light or phase-contrast microscope at 200x and 400x magnification to detect mycelial hyphae or spores (ZEISS, Germany) and OLIMPUS BX43 (Japan microscopes). Axio Scope A1 software (Zeiss, Germany) was used for microscopic visualization and data archiving.

The collected material was analyzed under a Wood's lamp. After a perforation test, the hair was processed and examined using light microscopy [27].

Cultivation of diagnostic material for fungal species identification was performed on ready-to-use Sabouraud dextrose agar (HiMedia Laboratories Pvt Ltd, India) at 28°C and 37°C for 1-3 weeks. Morphological identification of dermatomycetes was performed based on macroscopic and microscopic features visible in the obtained culture (characteristic hyphae and spores) using classical mycological criteria, including colony morphology, pigmentation, and microscopic characteristics [28].

Molecular genetics identification of micromycete isolates was performed using a standard method. PCR amplification of the variable *ITS* region of rDNA was performed using universal primers *ITS1* (5' TCCGTAGGTGAACCTGCGG3') and *ITS4* (5' TCCTCCGCTTATTGATATGC3'). PCR analysis results were interpreted by gel electrophoresis and documented using a GelDoc XR+ transilluminator (Bio-Rad, Hercules, CA, USA). Sequencing was performed on a SeqStudio genetic analyzer (Thermo Fisher Scientific Applied Biosystems, USA) [29].

Keratinophilic activity was assessed using a modified Sabouraud medium enriched with 2% keratin, and carotinolytic properties were assessed in a hair perforation test [28, 30]. Saccharolytic activity was studied on Giess media [31] containing lactose, glucose, sucrose, maltose, and mannitol. urease activity was assessed on Christensen medium with 40% urea [31], and proteolytic activity was assessed on media enriched with peptone, gelatin, skim milk, and sheep blood. To assess the hemolytic activity of fungi, Columbia nutrient medium with 5% sheep blood agar was used [32]. Caseinolytic activity of isolates was determined on skim milk agar medium after incubation of strains in Petri dishes at 28°C for 10 days. The transparent zone surrounding fungal colony was used as an indicator of the strains' ability to digest casein, which was measured using a ruler [33]. Edible gelatin was used to determine proteolytic activity based on gelatin liquefaction [34].

3. RESULTS

Of the 760 samples tested, 281 fungal isolates representing a broad taxonomic spectrum were obtained. The fungal community consisted of several diverse genera, including *Alternaria*, *Aspergillus*, *Aureobasidium*, *Botryotrichum*, *Chaetomium*, *Chrysosporium*, *Cladosporium*, *Penicillium*, *Rhizomucor*, *Trichoderma*, and *Trichothecium*. *Aspergillus* spp. constituted the majority of isolates, accounting for 60.9%.

Classic dermatophytes of the genera *Trichophyton* and *Microsporum* accounted for 6.2% of the isolates. Fungi of the genera *Alternaria*, *Chrysosporium*, *Mucor*, and *Penicillium* were represented by more than two isolates. Most strains were isolates occurring in two or more replicates. Given the objective, these strains were not further considered.

Several mold species were encountered in isolated cases: *Chaetomium*, *Trichoderma*, *Trichothecium*, *Botryotrichum*, and *Cladosporium*. To confirm or refute the pathogenicity of the isolates, their biological properties were studied. Among the rare isolates with high keratinophilic, proteolytic, or saccharolytic activity were strains of *Chaetomium*, *Trichoderma*, and *Trichothecium*.

Analysis of the cultural and morphological properties indicated the presence of characteristic cultural features inherent to the corresponding genus (Figure 1).

ing creamy pink with age, with a light peach reverse center.

Microscopic analysis revealed the characteristic morphological structures of the micromycetes (Figure 2).

As Figure 2 shows, *Trichoderma citrinoviride* has ellipsoidal, colorless conidia with septate hyphae. *Trichothecium roseum* is characterized microscopically by a hyaline (colorless), septate mycelium with straight, elongated conidiophores that form pear-shaped, two-celled conidia. Under the microscope, *Chaetomium globosum* has clearly visible coiled setae and ascospores. The ascospores are abundant, spherical, easily visible, and brown in color.

Analysis of the saccharolytic, urease, and proteolytic activities of these strains revealed significant differences in some enzymes, including those responsible for pathogenic properties. T. citrinoviride No. 20.25.51G exhibited the highest activity in the breakdown of carbohydrates: glucose (4

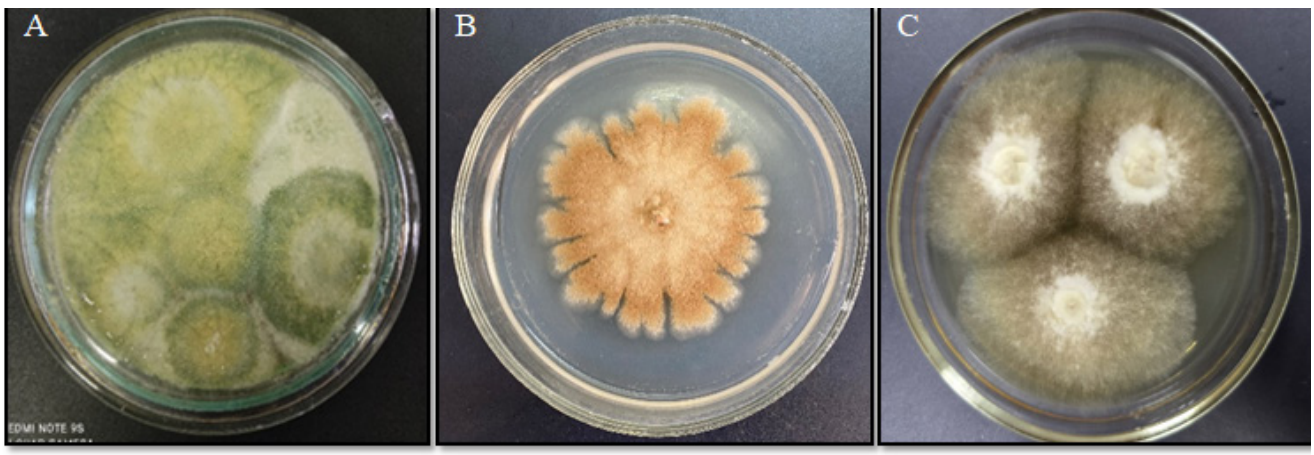


Figure 1 – Cultural properties of isolates of saprophytic fungi isolated from farm animals: A – *Trichoderma* spp., B – *Trichothecium* spp, C – *Chaetomium* spp.

As Figure 1 shows, *Trichoderma citrinoviride* colonies are characterized by the formation of abundant aerial mycelium with yellow-green spore pads, which become visible on the third day of cultivation. The release of an intense yellow pigment into the nutrient medium was noted. During cultivation, a faint moldy odor emanating from the plates was detected on days 3-5, intensifying over time.

Chaetomium globosum formed fluffy, grayish-green colonies with a light gray center, with a deep green reverse center.

Trichothecium roseum formed small, powdery, fast-growing colonies (1.8-2.4 cm in diameter), initially white, turn-

points), sucrose and lactose (2 points), urea (2 points), and hemoglobin (3 points). It also exhibited nor activity against mannitol and casein (1 point, respectively) (Figure 3).

Trichothecium roseum No.20.25.27G, isolated from a goat, was characterized by high urease activity (4 points). It had moderate saccharolytic activity (2 points) for the breakdown of maltose and sucrose disaccharides. It also had weak activity for glucose, mannitol, hemoglobin, and casein (1 point). No lactose- or gelatin-degrading enzymes were detected in *Trichothecium roseum* (Figure 4).

Two *Chaetomium globosum* strains were found to lack

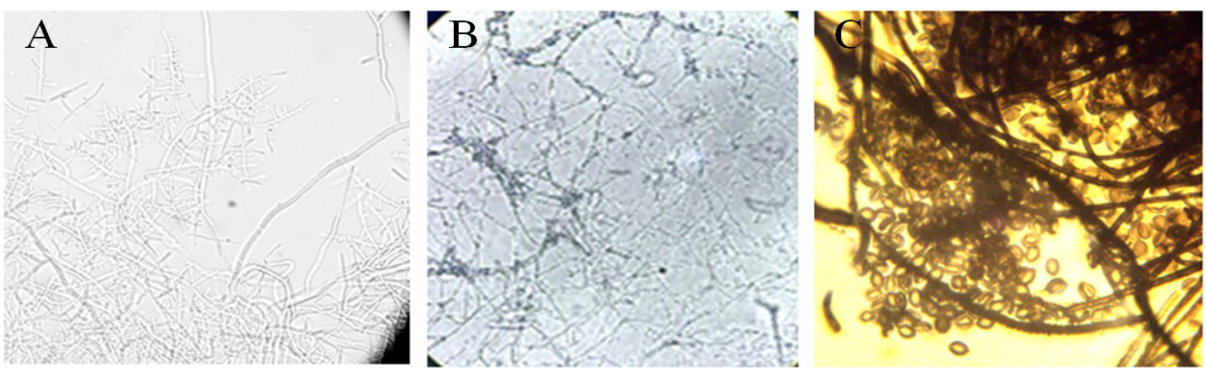


Figure 2 – Micromorphological properties of strains of saprophytic fungi isolated from farm animals, 40×: A – *Trichoderma citrinoviride;* B – *Trichothecium roseum; C – Chaetomium globosum.*

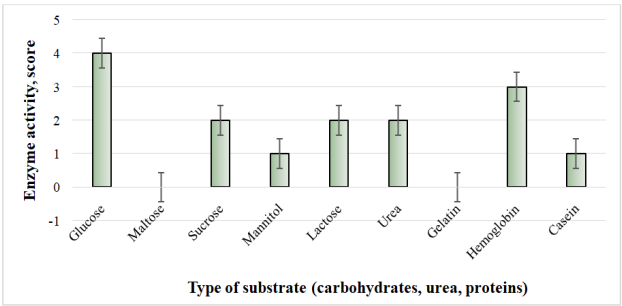


Figure 3 – Enzymatic activity of *Trichoderma citrinoviride* isolate No. 20.25.51G, isolated from farm animals.

the ability to break down glucose, maltose, lactose, gelatin, and hemoglobin. Strain *Ch. globosum* No.10.24.22H, isolated from a horse, demonstrated moderate activity toward sucrose, mannitol, and urea (2 points each). Strain *Ch. globosum* No.8.25.30.1Sh, isolated from a sheep, demonstrated enzymatic activity toward urea and casein (3 points), and moderate activity toward mannitol and hemoglobin (2 points) (Figure 5).

When analyzing the keratinophilic properties, it was revealed that the *Chaetomium globosum* strain No.10.24.22H demonstrated pronounced growth on a keratin-containing medium - 5.2 mm compared to Sabouraud's medium - 3.55 mm, and the growth of the *Chaetomium globosum* strain No. 8.25.30.1Sh was observed on a medium with keratin - 2.35 mm, on Sabouraud's medium - 4.65 mm, which indicates the inhibitory effect of keratin and a visible decrease in colony growth is observed. Strains *Trichothecium roseum* No.20.25.27G and *Trichoderma citrinoviride* No.20.25.51G practically did not assimilate keratin, which explains the equal

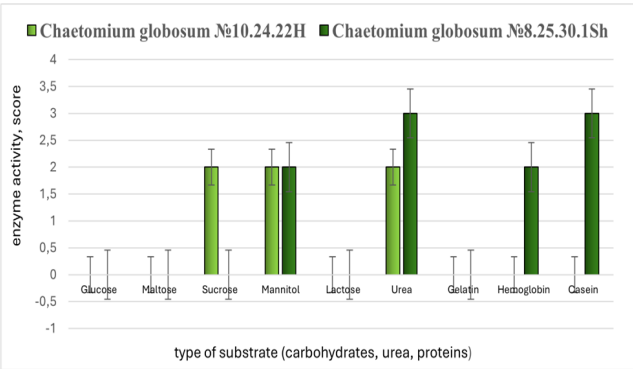


Figure 5 – Enzymatic activity of two isolates of *Chaetomium globosum* obtained from farm animals.

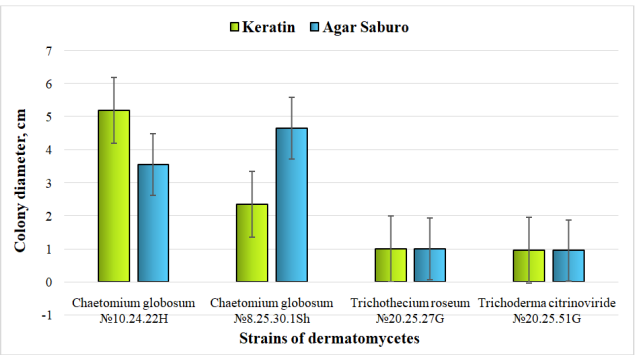


Figure 6 – Keratinophilic properties of saprophytic fungal isolates obtained from farm animals.

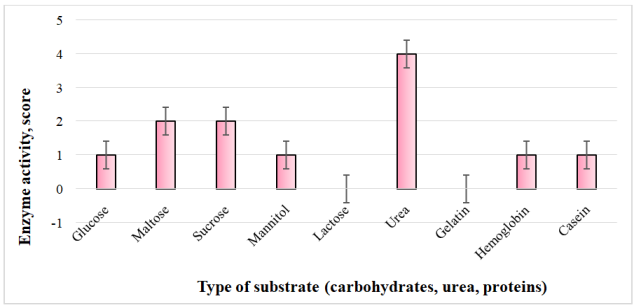


Figure 4 – Enzymatic activity of *Trichothecium roseum* isolate No.20.25.27G, isolated from farm animals.

colony diameter of 1 mm and 0.95 mm, respectively, on both media, standard Sabouraud agar and modified Sabouraud agar enriched with keratin (Figure 6).

The molecular genetics identification of fungal isolates was performed by sequencing the internal transcribed spacer (ITS) region of ribosomal DNA (Table 1).

The hair perforation test revealed keratinolytic activity in all isolates, manifested to varying degrees (Figure 7).

For *Chaetomium globosum* strains No.10.24.22H and No.8.25.30.1Sh, the test result was manifested by the destruction and detachment of the cuticle (1), melting and thinning of the hair (2), accumulation of mycelium on the hair (3), and the formation of clearly visible spherical brown ascospores (4). The most noticeable indicator was the destruction of the cuticle and the accumulation of biomass. *Trichotheciumroseum* strain No.20.25.27G actively destroyed the hair, melting it lengthwise with the formation of uncharacteristic «pegs» (5), and accumulating a large amount of biomass (3). The *Trichoderma citrinoviride* strain No. 20.25.51G accumulated biomass, forming olive-colored mycelial strands (6), and caused focal hair lesions (7), forming characteristic "pegs".

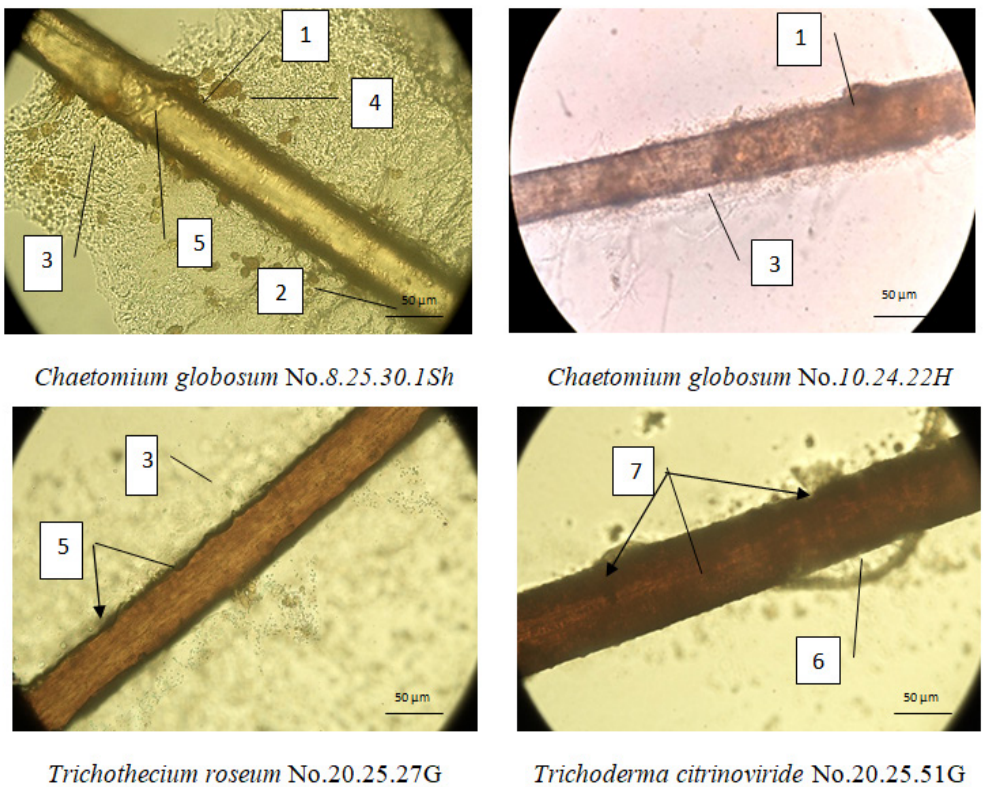
4. DISCUSSION

In recent years, there has been an increase in the incidence of cutaneous mycoses caused by non-dermatophyte molds and rare yeasts [35]. These microorganisms have traditionally been considered environmental saprophytes; however, accumulating data indicate their ability to cause opportunistic infections of the skin and its appendages in humans and animals, especially in cases of immunocompromise, skin trauma, or prolonged exposure to a moist environment. In Europe, the most frequently identified non-dermatophytes are *Scopulariopsis brevicaulis*, *Aspergillus* spp., *Acremonium* spp., and *Fusarium* spp., with *Fusarium* spp. accounting for 7.5% to 9.2% of onychomycosis cases. In tropical regions such as Thailand, *N. dimidiatum* is the major pathogen, accounting for 13% of onychomycosis cases [36]. A study by Rahimi and Mohammadi (2020) demonstrated that non-dermatophyte molds, including members of the genera *Aspergillus*, *Penicillium*, *Fusarium*, and others, may constitute a significant proportion of isolates of suspected dermatomycosis in animals. The authors emphasize that these microorganisms are capable of colonizing affected tissues and, in some cases, act as etiologic agents of dermatomycosis, which complicates diagnosis and requires laboratory confirmation [3].

Our results on the species assignment of the identified micromycetes isolated from biomaterial from affected areas and

Table 1 – Molecular genetics identification of isolates.

#	Type Sequence	Sequence
1	*Chaetomium globosum* No.10.24.22H	ACCATTGTGAACGTTACCTATACCGTTGCTTCGGCGGGCGGCCCCGGGGTTTAC CCCCCGGGCGCCCCTGGGCCCCACCGCGGCGCCCGCCGGGTCACCAAACTCT TGATAATTTATGGCCTCTCTGAGTCTTCTGTACTGAATAAGTCAAAACTTTCAA CAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAG TAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGC CCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCATCAAGC CCCCGGGCTTGTGTTGGGGACCTGCGGCTGCCGCAGGCCCTAAAAGCAGTGG CGGGCTCGCTGTCGCACCGAGCGTAGTAGCATACATCTCGCTCTGGTCGCGCC GCGGGTTCGGCCGTTAAACCACCTTTTAACCCAAGGTGACCCTCGATTCAGGT AGGAAGACCCGCTGAACTAAGCATATCAATAAG
2	*Chaetomium globosum* No.8.25.30.1Sh	CTCTTGTTAATTTATGGCCTCTCTGAGTCTTCTGTACTGAATAAGTCAAAACTTT CAACACGGGATCTCTTGTTCTGGCATCGATGGAAGAACGCAGCGGAAATGCGA TAAGTATATGTGAATGCAAGAATTCAGTGATCATTCGATCTTTGAACCGCACAT TGCGCCCGCCAGTATTTCGGCGGGCAGTGCTTGTTCAGC
3	*Trichothecium roseum* No.20.25.27G	CCCTTTGTGAACCTTACCTACCGTTGCTTCGGCGGACCGCCCCGGGCGCTGCG TGCCCCGGACCCAAGGCGCCCGCAGGGACCACACGAACCCTGTTTAACAAAC ATGTGTATCCTCTGAGCGAGCCGAAAGGTTCAAAACAAATCAAAACTTTCAAC AACGGATCTCTTGGTCTGGCATGATGAGAACGCAGCGAAATGCGATAAGTAAT GTGAATTGAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGC CGGTATTCTG
4	*Trichoderma citrinoviride* No.20.25.51G	GGGCCATGCTGTCGAGCGTCATTCAACCCTCGGGACCCCCTTTCCCTGCGGGA GGGCGGTAGGTGGGCAGCTCTCAGGGCTTCAGAACCATGGGTTGCGGCCCTC GGTGTAAAACCTGGGGGGGGAGGGGGGGGCCACCCTAAAAACCCAAATTTAT CAAGTTGAACTTCGATTAGAGAGAAACCCCCTGAATTAGGATACAATAGGAGA AGAAAAATGTCACTAAGCCGAAGAAGGGTTAGGGG



Chaetomium globosum No.*8.25.30.1Sh* *Chaetomium globosum* No.*10.24.22H*

Trichothecium roseum No.20.25.27G *Trichoderma citrinoviride* No.20.25.51G

Figure 7 – Results of hair perforation test with isolates of saprophytic fungi isolated from farm animals.

the fur of farm animals are consistent with data from veterinary and medical mycologists reporting a shift in the spectrum of dermatomycosis pathogens. In our studies, fungi of the genus *Aspergillus* spp. accounted for the majority of isolates – 60.9%, dermatophytes of the genera *Trichophyton* and *Microsporum* – 6.2%, fungi of the genera *Alternaria*, *Chrysosporium*, *Mucor*, and *Penicillium* were represented by 1.5 to 4%.

Along with well-known and frequently isolated genera of opportunistic pathogens, rare species of pathogens are described in the literature, most often considered ecologically significant keratinophilic saprophytes or phytopathogens. From the point of view of the «One Health» concept, a number of rare fungi may have zoonotic potential. Kundu et al. (2024) emphasize that pathogenic fungi circulate between humans, animals, and the environment, which makes it important to study rare and opportunistic species as possible sources of infection [24]. Considering that micromycetes *Chaetomium* spp., *Trichoderma* spp., *Trichothecium* spp. Isolated from animal skin lesions, we analyzed the pathogenicity factors of each opportunistic micromycete.

Fungi of the genus *Chaetomium* are of particular interest. Although they are widespread in soil and plant debris, cases of their involvement in skin and nail diseases have been described.

Fungi of the genus *Chaetomium* are known for their pronounced ability to degrade keratin-containing substrates, which has been confirmed by experimental and clinical studies. A member of the genus, *Chaetomium globosum*, has been described as a potential causative agent of skin lesions, nail plates, and keratinized structures in humans and animals. The key mechanism of its pathogenicity is its high keratinophilic activity, which allows this micromycete to effectively colonize the stratum corneum [37]. Kim et al. (2013) reported a clinical case of onychomycosis caused by *Chaetomium globosum*. The infection manifested as dystrophy of the nail plate, and the identification of the pathogen was confirmed by cultural and microscopic methods [15]. Hubka et al. (2011) described several cases of phaeohyphomycosis and onychomycosis caused by *Chaetomium* species, including the first report of infection with *Ch. brasiliense*. The authors note that these fungi can act as opportunistic pathogens, especially when the nail plate or skin is damaged [18]. Zhang et al. (2010) reported skin infections caused by *Chaetomium atrobrunneum* and the yeast-like fungus *Clavispora lusitaniae*. Both pathogens are considered rare opportunistic pathogens capable of causing chronic skin lesions [16].

For *Chaetomium* spp. the main pathogenic factors for animals include thermotolerance, which allows strains to grow at 37°C and above, making it a dangerous opportunistic pathogen (it can cause onychomycosis and even brain abscesses). *Chaetomium* spp. produces cytotoxins that disrupt host cell division and can suppress macrophage activity; it has adhesive properties, as a result of which it adheres well to the keratin of nails and skin [38, 39].

During our studies, we identified isolated cases of isolating representatives of the genus *Chaetomium* from animals. Thus, an isolate of the strain *Ch. globosum* No.8.25.30.1Sh with high activity towards urea and casein, and moderate activity towards mannitol and hemoglobin was isolated from a sheep. The strain did not possess keratinophilic properties, but had pronounced keratinolytic properties. *Ch. globosum* No.8.25.30.1Sh caused hair damage, breakage and detachment Cuticle, abundant growth of mycelial mass along the hair length, accompanied by the formation of clearly visible spherical ascospores. *Chaetomium globosum* strain No.10.24.22H, isolated from a horse, possessed keratinolytic and keratinophilic properties, actively breaking down sucrose, mannitol, and urea, disrupting the integrity of the cuticle, thinning the hair and accumulating mycelial mass.

Thus, both isolates had pronounced keratinophilic activity. This is consistent with literature data on the ability of *Chaetomium* species to actively degrade keratin substrates. High growth rates on Sabouraud medium with keratin from *Chaetomium globosum* strain No. 10.24.22H also confirm its good adaptation and metabolic activity. This isolate exhibited virtually no proteolytic properties, but did demonstrate a high level of urea degradation. This indicates a narrow enzymatic focus, primarily on keratinophilic mechanisms and adaptation to skin habitats, where sweat glands provide the micromycetes with a feeding substrate. *C. globosum* demonstrated a good ability to ferment mannitol, which underscores its aggressiveness as a pathogen, as its ability to cleave mannitol is one of the pathogenicity factors.

Trichoderma fungi are traditionally known as soil saprophytes and biocontrol agents. However, Zhang and Li (2022) demonstrated that *Trichoderma longibrachiatum* is capable of inducing inflammatory skin changes and atypical hyperplasia in an experimental mouse model. These data confirm the potential of this species as an opportunistic dermatotropic pathogen [17]. Fungi of the genus *Trichoderma* are characterized by pronounced proteolytic activity and the ability to synthesize a wide range of hydrolytic enzymes. Due to these properties, they are highly competitive in microbial communities and can actively participate in the development of inflammatory processes in the skin, especially during secondary colonization of damaged tissues. Although *Trichoderma* spp. are traditionally considered saprotrophs and biological antagonists of phytopathogens, in recent years evidence has emerged of their involvement in the development of opportunistic mycoses in animals and humans [40], which is confirmed by the isolation of *T. citrinoviride* from a patient's toenail [41].

For *Trichoderma citrinoviride*, the main pathogenic factor is indicated to be the ability to grow at 37°C, which allows strains to survive and sporulate [42]; secretion of hydrolytic enzymes chitinase, β-glucanase and protease; production of secondary metabolites; Rapid growth and substrate colonization [43].

Isolate No.20.25.51G, isolated from a goat and identified as *Trichoderma citrinoviride*, possessed several characteristics indicative of pathogenicity. *T. citrinoviride* No.20.25.51G is characterized by high activity in the breakdown of carbohydrates (maltose, sucrose, lactose, urea, and hemoglobin), and insignificant activity against mannitol and casein. Despite the lack of pronounced keratinophilic properties, we detected keratinolytic activity. Focal lesions of the cuticle, the formation of mycelial strands, and melting of the hair shaft with the formation of typical «pegs» are clearly visible on the hair. Therefore, the description of cases similar to ours, in which *T. citrinoviride* was isolated from keratin-containing tissues, the presence of enzymatic activity of certain proteases and other hydrolytic enzymes, and the ability to rapidly grow and colonize hair, indicate the pathogenic potential of our isolate.

Another potential agent of skin infections is *Trichothecium roseum*, a mold commonly found in the environment. Although clinical infections are rare, its biological properties and ability to produce mycotoxins indicate possible pathogenicity for humans [22, 44].

Pathogenicity factors of *Trichothecium* spp. include the ability to form toxins, possess proteolytic activity, and the ability to actively sporulate. *Trichothecium* spp. is known to synthesize trichothecenes, which are inhibitors of protein synthesis. They cause profound tissue damage, suppress the immune system, and can lead to necrosis. *Trichothecium* spp. also secretes enzymes that degrade extracellular matrix proteins, facilitating tissue penetration. The massive production of *Trichothecium* spp. spores in the lungs facilitates their deep penetration into the respiratory tract, causing allergic alveolitis or pulmonary mycosis [45, 46].

We isolated only one isolate of *Trichothecium roseum* No.20.25.27G from a goat, which exhibited high enzymatic activity, degrading virtually all substrates, especially urea. Despite the virtual absence of visible keratinophilic activity in a Petri dish, the strain actively degraded hair, melting it to form uncharacteristic «pegs,» and accumulating a large amount of biomass containing spores.

The study compared the biochemical properties of isolates of the micromycetes *Chaetomium* spp., *Trichoderma* spp., and *Trichothecium* spp., which are considered rare. Rare micromycetes isolated from animal skin exhibit varying enzymatic properties. The results revealed significant differences between species in keratinophilic, proteolytic, and saccharolytic activity. At the same time, common characteristics of the biochemical activity of rare micromycetes were established. All strains actively degraded urea, degraded hemoglobin and casein to varying degrees, did not degrade gelatin, and were capable of assimilating mannitol. All exhibited high levels of keratinolytic activity.

CONCLUSION

The study demonstrates significant variability in enzymatic properties among rare filamentous fungi of the genera *Chaetomium* spp., *Trichoderma* spp., and *Trichothecium* spp. isolated from the skin of farm animals. Of the four isolates studied, one strain, *Chaetomium globosum* No.10.24.22H, demonstrated the most pronounced keratinophilic activity, with a colony diameter of 5.2 mm on keratin-containing medium compared to 3.55 mm on Sabouraud medium. *Trichoderma citrinoviride* demonstrated glucose degradation activity of 4 points, sucrose and lactose (2 points), and urea and hemoglobin (2 and 3 points, respectively). It demonstrated minor activity against mannitol and casein and exhibited pronounced keratinolytic properties. *Trichothecium roseum* exhibited high enzymatic activity, decomposing virtually all substrates, especially urea (4 points); it actively destroyed hair, melting it to form uncharacteristic «pegs,» and accumulating a large amount of biomass with spores.

CONTRIBUTION OF THE AUTHORS

Conceptualization, Y.K; formal analysis, Y.K, G.B; resources, Y.K; preparation of original draft, Y.K; and writing review and editing, Y.K, T.G, P.R. All authors have read and agreed with the published version of the manuscript.

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CONFLICT OF INTEREST

There are no conflicts of interest to declare.

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ПАТОГЕННЫЙ ПОТЕНЦИАЛ РЕДКИХ ВОЗБУДИТЕЛЕЙ ОППОРТУНИСТИЧЕСКИХ МИКОЗОВ КОЖИ, ВЫДЕЛЕННЫХ ОТ ЖИВОТНЫХ В КАЗАХСТАНЕ

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АБСТРАКТ

В последние десятилетия наблюдается изменение спектра возбудителей с увеличением доли оппортунистических и редких грибов, участвующих в развитии инфекций кожи у животных. Целью настоящего исследования явилось изучение биологических свойств редких условно-патогенных плесневых грибов, выделенных с кожи сельскохозяйственных животных в Казахстане. В ходе работы было исследовано 760 образцов биоматериала, отобранных у крупного рогатого скота, овец и лошадей в шести регионах Северного и Центрального Казахстана. В результате микологического анализа выделен 281 изолят грибов, среди которых выявлены редкие представители родов *Chaetomium*, *Trichoderma* и *Trichothecium*. Идентификация изолятов проводилась с использованием культурально-морфологических и молекулярно-генетических методов. Изучены ферментативная (протеолитическая, уреазная, сахаролитическая), кератинофильная и кератинолитическая активности штаммов. Установлено, что редкие изоляты обладают выраженным ферментативным потенциалом, связанным с факторами патогенности. *Chaetomium globosum* характеризовался высокой кератинофильной и кератинолитической активностью. *Trichoderma citrinoviride* проявлял высокую активность в расщеплении углеводов и умеренную кератинолитическую активность. *Trichothecium roseum* отличался высокой общей ферментативной активностью и способностью активно разрушать волос.

Ключевые слова: *Chaetomium*; *Trichoderma; Trichothecium;* микозы кожи; плесневые грибы; редкие возбудители; ферментативная активность.

ҚАЗАҚСТАНДА ЖАНУАРЛАРДАН БӨЛІНГЕН ОППОРТУНИСТІК ТЕРІ МИКОЗДАРЫНЫҢ СИРЕК ҚОЗДЫРҒЫШТАРЫНЫҢ ПАТОГЕНДІК ӘЛЕУЕТІ

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ТҮЙІН

Соңғы он жылдықтарда жануарлардың тері инфекцияларының дамуына қатысатын оппортунистік және сирек кездесетін саңырауқұлақтардың үлесінің артуымен қоздырғыштар спектрінің өзгеруі байқалды. Осы зерттеудің мақсаты Қазақстанда ауыл шаруашылығы жануарларының терісінен бөлінген сирек кездесетін шартты-патогенді зең саңырауқұлақтарының биологиялық қасиеттерін зерттеу болды. Жұмыс барысында Солтүстік және Орталық Қазақстанның алты өңірінде ірі қарамалдан, қой мен жылқы даналынған биоматериалдың 760 үлгісі зерттелді. Микологиялық талдау нәтижесінде саңырауқұлақтардың 281 изоляты анықталды, олардың арасында *Chaetomium*, *Trichoderma* және *Trichothecium* тұқымдарының сирек өкілдері анықталды. Изоляттарды анықтау өсінділік - морфологиялық және молекулалық-генетикалық әдістерді қолдану арқылы жүргізілді. Штаммдардың ферментативті (протеолитикалық, уреазиялық, сахаролитикалық), кератинофильді және кератинолитикалық белсенділігі зерттелді. Сирек изоляттардың патогендік факторлар мен байланысты айқын ферментативті потенциалы бар екендігі анықталды. *Chaetomium globosum* жоғары кератинофильді және кератинолитикалық белсенділікпен сипатталды. *Trichoderma citrinoviride* көмірсулардың ыдырауында жоғары белсенділікті және орташа кератинолитикалық белсенділікті көрсетті. *Trichothecium roseum* жалпы ферментативті белсенділігімен және шашты белсенді түрде жою қабілетімен ерекшеленді.

Түйін сөздер: *Chaetomium*; *Trichoderma*; *Trichothecium*; терімикоздары; зеңдер; сирек қоздырғыштар; ферментативті белсенділік.