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Research article

Isolation and characteristics of keratinophilic fungi from the objects of the external environment

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Abstract

Background and Aim. There is a growing number of fungal skin infections in animals worldwide. The frequent detection of mold fungi in association with dermatomycetes, or as a mono infection, indicates exogenous sources of infection.

The aim of this study is to isolate and characterize keratinophilic fungi from soil and other environmental sources.

Materials and Methods. A total of 198 environmental samples (soil, litter, feed, scrapings from feeders, fences, paddocks, and walls) were collected from Akmola, Almaty, Karaganda, and Pavlodar regions of Kazakhstan. Hair bait technique was used to isolate keratinophilic fungi. Cultural and morphological features were studied on universal dense agar and microscopically at ×10 and ×40 magnifications. Biochemical properties were determined using Hiss media, Christensen medium with 40% urea, and media with milk and gelatin.

Results. Ecologically significant saprophytic keratinophilic micromycetes were identified in the samples: *Trichophyton* spp. – 7%, *Phoma* spp. – 7%, *Marquandomyces* spp. – 7%, *Penicillium* spp. – 13%, *Lecanicillium* spp. – 7%, *Fusarium* spp. – 7%, *Alternaria* spp. – 7%, *Aspergillus* spp. – 6%, *Filobasidium* spp. – 13%, *Mucor* spp. – 26%.

Among isolates, 85% utilized sucrose, 77% maltose, 69% glucose, and 62% mannitol. Urease activity was observed in 70%, and protein-degrading enzyme activity (gelatin and casein) in 50% of isolates. All strains showed varying degrees of keratin assimilation. The most severe hair damage was caused by *Trichophyton* spp., *Penicillium* spp., *Alternaria* spp., and *Aspergillus* spp., which formed dense mycelial sheaths or conidia, leading to thinning and lysis of hair.

Conclusion. The wide distribution of saprophytic keratinophilic fungi in the environment suggests that animal infections are caused not only by classical dermatophytes like *Trichophyton*, but also by opportunistic keratin-degrading molds.

Keywords: dermatophytosis; keratin; keratin hydrolysate; keratinophilic fungi; environmental objects; soil.

Introduction

Currently, there is an active increase in the number of fungal skin infections in animals worldwide. It is believed that this is due to climate change, anthropogenic impacts, and disruptions of the ecosystem – soil, water, and other objects that are natural reservoirs of fungal spores [1, 2]. Dermatophytosis is

the most common fungal infection in animals and is caused by dermatophytes – the filamentous fungi *Microsporum* spp., *Trichophyton* spp., *Nannizzia* spp., *Arthroderma* spp. and a number of other recently added genera [3, 4]. Dermatophyte-induced diseases are widespread worldwide. The annual number of fungal infections increases not only in wild and farm animals but also in humans and pets [5].

Y.N. Haggag et al. (2017) identified dermatophytes in 150 farm animals, including 50 cattle, 25 buffalo, 50 sheep, and 25 horses. In young animals (up to 2 years old), the infection rate was 38.76% in children and 35.33% in adults. In horses, dermatophytosis was more common in males (44%), whereas in other species, it was more common in females. The high incidence rate is explained by contact transmission, long-term viability of spores in the environment, stress factors (overcrowding, poor sanitation), seasonality (more common in winter), and weak immunity in young animals. In the study, they identified the following dermatophytes: *Trichophyton verrucosum* (47.33%) was the most common, isolated from cattle (48%), buffalo (44%), sheep (64%) and horses (16%); *T. mentagrophytes* (14%) was isolated from cattle (18%), buffalo (32%), sheep (4%) and horses (8%); *T. equinum* (7.34%) was isolated exclusively from horses (44%); and *Microsporum canis* (5.33%) was isolated from cattle (6%) and sheep (10%) [6].

A.P.N. Albano et al. (2012) studied 30 wild cats divided into two groups: 7 cats kept in temporary captivity and 23 caught in the wild. Among them were 11 pumas, 9 Geoffroy's cats, 4 margays, 2 ocelots, 2 tiger cats, 1 jaguarundi, and 1 jaguar. Dermatophytes were found in two animals: a Geoffroy's tiger cat in its ear canal (*T. mentagrophytes*) and a jaguar (infected by an unspecified species of *Trichophyton* spp.) The main cause of infection is probably contact with rodents that wild cats feed on, which confirms the role of these animals as possible asymptomatic carriers capable of spreading the infection among other animals and humans [7]. A study aimed at analyzing the mycobiota of the skin and hair of domestic animals with an emphasis on the role of dermatophytes as a potential source of infections for humans and animals showed that dermatophytes were present in 44.1% of cats and 43.8% of dogs. The most frequently detected species were *M. canis* (17.7% in cats) and *T. mentagrophytes* (23.5% in cats, 25% in dogs). In some cases, dermatophytes were detected in combination with yeasts such as *Candida albicans* and *Rhodotorula mucilaginosa*. Detection of the dermatophytes *M. canis* and *T. mentagrophytes* on the skin and hair of animals indicates a high probability of infection from the external environment, which serves as a natural reservoir for these microorganisms [8]. Fungi play a key role in ecosystems as natural organic matter recyclers that return nutrients to the soil [9]. Soil, air, water, organic materials, and other environmental objects are sources of fungi, including ecologically significant and pathogenic species [10]. Fungi present in various natural environments colonize and decompose animal remains rich in keratin, a protein with high nitrogen and sulfur content. In the soil, keratin from keratinized remains of the skin and its appendages is decomposed by saprophytic microorganism - biodestructors that synthesize the enzyme keratinase, such as *Chrysosporium* spp., *Pseudogymnoascus* spp., *Geomyces* spp., *Pectinotrichum* spp., *Renispora* spp., and a number of others [11, 12]. *Aspergillus*, *Penicillium*, *Fusarium*, *Microsporum*, *Trichoderma*, and *Chrysosporium* are the most common keratinolytic fungi [13]. The surface and deep soil layers are the largest reservoirs and natural habitats of these keratinophilic fungi, as they contain keratin, most often in the form of mammalian hair (mainly rodents), bird feathers, claws, horns, and a number of other keratinized animal remains [14, 15].

From 40 soil samples collected from urban waste and pastures in Kanpur, 83 fungal species were isolated, among which *Chrysosporium*, *Microsporum*, *Trichophyton*, and *Aspergillus* were predominant. The authors found that urban waste and pasture soils are rich sources of keratinophilic fungi, including potential pathogens, highlighting their role in waste biodegradation and associated health risks [16].

Studying the ability of fungi isolated from soil to degrade chicken feathers and human hair, J. Kumar et al. (2020) revealed the keratinolytic ability of 11 strains of keratinophilic fungi. The results showed active keratin substrate degradation with the formation of perforating structures and chemical changes, which were particularly noticeable in *Chrysosporium indicum* and *Ch. tropicum* [17].

Isolation and identification of keratinophilic fungi from livestock barn soils in Cayenne, South Khorasan Province, Iran, using the hair bait method and molecular methods from 62 samples revealed 118 fungal isolates belonging to 7 species from 5 genera. The predominant species was *Aphanoascus verrucosus* (59.36%), followed by *Arthroderma quadrifidum*, *A. terreus*, *Acremonium* spp., *A. gertleri*, *Fusarium equiseti*, and *Uncinocarpus reesii* [18].

When analyzing the mycobiota of oil-contaminated soils and the surface air layer in the Binagadi district of Baku, it was found that oil pollution contributes to an increase in the species diversity of micromycetes. The authors identified 34 fungal species from 10 genera in the soil and 25 species from 8 genera in the surface air layer. The genera *Aspergillus*, *Penicillium*, *Cladosporium*, and *Fusarium* occupied the main position. For species such as *A. fumigatus* and *A. alternata*, increased rates of development and sporulation were observed [19]. Commonly found in water systems are genera of pathogenic fungi, such as *Aspergillus* spp., *Penicillium* spp., *Candida* spp., *Fusarium* spp., and *Trichoderma* spp. These organisms can contaminate water supplies, posing a serious health threat, especially to immunocompromised animals and humans [20].

In rural Ismailia, Egypt, 15 keratinophilic fungi, including 8 *Chrysosporium* species, were isolated using the hair bait method. *C. zonatum* was mainly isolated from soil collected from fields, animal cages, and the hair of cows and buffaloes [21].

In India, nine fungal species belonging to 6 genera were isolated from the feather samples of 117 birds representing 11 species tested for keratinophilic fungi. Among these, four species were identified as members of the genus *Chrysosporium*: *Chrys. indicum* (26.4%), *Chrys. tropicum* (11.1%), and *Chrys. Aphanoascus* spp. (2.5%) and *Arthroderma tuberculatum* (3.4%) [22].

The frequent presence of mold fungi in pathological material in association with dermatomyces or the detection of mono infection in mold mycoses of the skin indicates exogenous sources of infection, emphasizing the importance of the ecological aspect in the epidemiology of fungal infections [23]. It is believed that anthropogenic pollution stimulates the functional activity of the soil mycobiota. This contributes to the transformation of saprotrophic fungi into opportunistic forms, increasing the risk of infections, especially in urban environments [5, 19].

When studying pathological material (hair, crusts) from affected skin areas of commercial sables, collected in 2018–2019 in various regions of Russia, 18 taxa of fungi were identified. Keratinophilic dermatophytes (*Arthroderma cuniculi*, *Chrys. carmichaelii*) and secondary opportunistic pathogens were found in 12% of the samples—including representatives of the genus *Aspergillus* spp. (36%), *Scopulariopsis* spp. (16%), and *Acremonium* spp. (14%) [24].

Analysis of biological material from domestic and wild animals in northern Kazakhstan showed that dermatomyces of the genera *Trichophyton* spp. and *Microsporum* spp. were detected in only 17.1% of cases, whereas opportunistic mold fungi of the genera *Mucor* spp., *Penicillium* spp., *Aspergillus* spp., *Alternaria* spp., *Chaetomium* spp., *Eurotium* spp., *Phoma* spp., *Trichoderma* spp., *Lecanicillium psalliotae*, *Scop. brevicaulis*, and others were present in 50.2%. Yeasts of the genera *Candida* spp., *Rhodotorula* spp., *Exophiala* spp. accounted for 5.1% of cases [25]. Analysis of pathological material samples from calves with clinical signs of dermatophytosis in the Almaty, Turkestan, and Kyzylorda regions of Kazakhstan indicated that the main causative agent of the disease was the fungus *Trichophyton* sp., which was identified using microscopic methods (in 86% of cases), microbiological methods with the isolation of a pure culture (in 79% of cases), and the polymerase chain reaction method, which showed the highest sensitivity (97.9% [26]. In the northern region of Kazakhstan, the most commonly identified etiological agent of dermatophytosis is *Trichophyton* spp. [27]. In Kazakhstan, fungal skin diseases are commonly observed in humans. In eastern Kazakhstan, among the causative agents of dermatomycosis in humans, *Microsporum* spp., *Trichophyton* spp. the frequency of sowing increasing [28]. In 2022, in the Kostanay region, fungal skin diseases affected 51 people. In the first half of 2022 in Kyzylorda, 76 people were infected with dermatophytosis. In Almaty, in 2023, the incidence of fungal skin diseases among people increased by about 1.5 times compared with the previous year. The highest incidence of dermatophytosis was recorded in children aged under 14 years (85% of all cases) [26].

The data we found during the analysis of the literature emphasize the relevance of soil monitoring for the presence of keratinophilic fungi and the need for prevention to reduce the risk of fungal infections.

The aim of this study was to isolate and identify keratinophilic fungi present in soil and other environmental objects.

Materials and Methods

The material consisted of 113 samples of environmental objects (EO): soil, litter, feed, scrapings from feeders, fences of pens, and walls from different regions of Kazakhstan—Akmola, Almaty, Karaganda, and Pavlodar regions.

To isolate keratinophilic fungi, soil samples were collected from a depth of 5 cm using a metal spatula and placed in wide-necked, sterile glass jars with a capacity of 500 ml. Scrapings were made from walls, fences, posts, and feeders in places where wool- or fat-like accumulations were found. The litter and contents of the feeders were collected using tweezers and packed in zip-lock bags. The feed remains were collected from the bottoms of the feeders, from storage areas at the soil boundary, and in the passages where the animals were fed [29].

The samples were poured into a glass jar and then spread in a 5-mm layer on the bottom of sterile Petri dishes, and hair baits were prepared. For this purpose, hair, previously cut into 3-cm-long pieces and autoclaved at 121 °C for 20 min, was evenly spread on the surface of the samples. Petri dishes wrapped in paper were placed in a dark place at 25 °C for 3-4 weeks, and the soil was periodically moistened as needed [30].

Sabouraud dextrose agar was used for the primary isolation of fungi and for obtaining a pure culture by the direct plate method. Samples of the biomaterial were placed on the surface of the agar medium using sterile tweezers. The Petri dishes were placed in a thermostat and incubated at a temperature of 28 °C. To isolate and identify keratinophilic fungi from EO, pieces of soil, feed, bedding, etc., were transferred to a Petri dish with sterile filter paper soaked in liquid Sabouraud medium, where traps for keratinolytic fungi were laid out in the form of children's or women's hair cut into pieces of 2–3 cm.

To identify the keratinophylic properties of the fungi, we used Sabouraud dextrose agar with the addition of 2% sterilized hair. The medium created favorable conditions for the growth of fungi with keratinolytic activity.

To study saccharolytic properties, Giss nutrient media with glucose, mannitol, lactose, sucrose, and maltose were used. The cultures were sown in test tubes using the prick method and incubated at 28 °C under constant observation for color changes, turbidity, and gas formation. To identify urease activity, we determined the ability of the fungi to decompose urea to ammonia on Christensen medium with the addition of 40% urea. Changes in the medium that occurred during cultivation were visually assessed, and the intensity of the reaction was expressed in crosses (from “+” to “++++”) [31].

The proteolytic activity of the fungi was studied in gelatin and skim milk. Meat-peptone agar with the addition of gelatin was used to assess the ability of fungi to hydrolyze gelatin [32]. Cow's milk, previously skimmed by centrifugation at 3000 rpm and 2 °C for 60 min, was used to study casein breakdown. Sowing was performed using the prick method in test tubes, after which the tubes were incubated in a thermostat at a temperature of 28 °C for 7 days.

The identification of the resulting cultures was carried out by considering colony growth and morphological characteristics. For microscopy, scotch tape preparations were prepared and examined under a light microscope at a magnification of $\times 40$. The identification of pathogens was carried out using identifiers [33].

Results and Discussion

Analysis of the isolation of fungi and bacteria from samples of EO showed that 57.01% of the samples were positive (Table 1).

Table 1 – Isolation of micromycetes from environmental objects

Sampling location, region	Number of samples	Including						Of which, positive
		soil	cage	feeders	litter	burrow	Feed	
Akmola	107	20	26	22	15	16	8	89
Karaganda	66	13	11	7	15	20	-	12
Pavlodar	15	4	5	1	4	-	0	1
Almaty	10	3	2	3	2	-	-	1
Total:	198	40	44	33	36	36	9	113

As shown in Table 1, the highest frequency of micromycete detection was noted in the Akmola (83.2%) and Pavlodar (73.3%) regions, and lower in the Karaganda (18.2%) and Almaty (10%) regions.

In the Akmola region, 107 samples of EO were examined. Among these, micromycete growth was observed in 89 cases (83.2%). In the Karaganda region, fungal growth was detected in 12 of 66 samples, representing 18.2%. In the Pavlodar region, in 33.3% of cases, no growth of micromycetes was noted. Of the 15 samples, in one case, growth of the micromycete *Mucor plumbeus* was noted, which amounted to 6.7%. At the same time, 10 bacterial strains (66.7%) were identified in the Pavlodar region. In the Almaty region, out of 10 samples, fungal growth was also observed in only one case (*Mucor plumbeus*), corresponding to 10% growth, with no growth in 90% of samples.

Analysis of the spectrum of the identified micromycetes made it possible to establish that the frequency of isolation of *Alternaria* spp. was 38.94%, followed by *Fusarium* spp. – 22.13%, *Mucor* spp. – 14.17%, *Penicillium* spp. – 8.85%. Bacteria were isolated in 8.85% of the cases. Less frequently, fungi of the species *Lecanicillium saksenae* and *Filobasidium magnum* were isolated (each at 1.77%). Other species, including *Aspergillus cristatus*, *Phoma livicola*, *Marquandomyces* spp., and *Trichophyton verrucosum*, were detected even less frequently (0.88% in each case) (Figure 1).

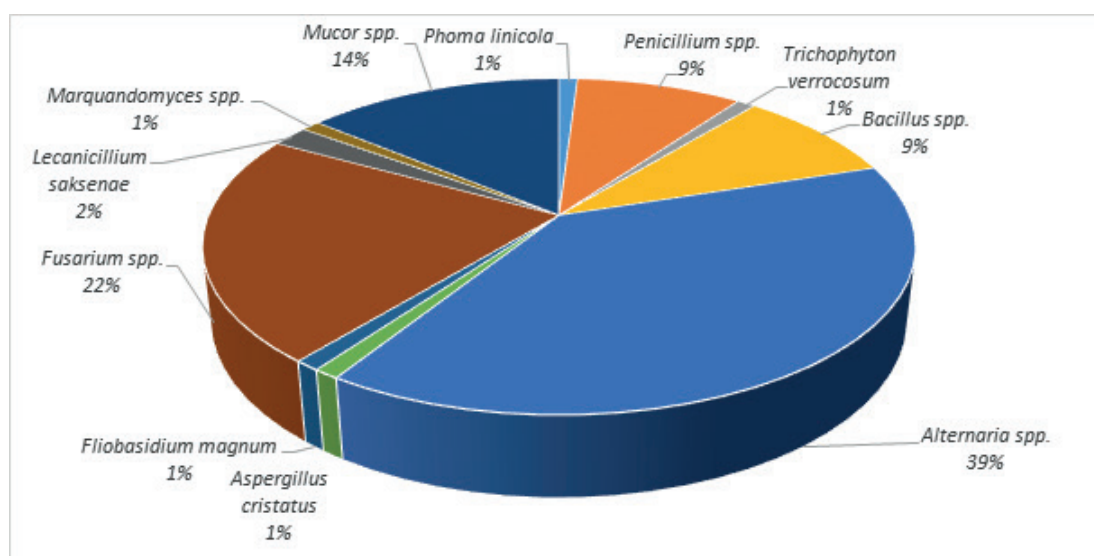


Figure 1 – Spectrum of microorganisms isolated from environmental objects

Cultivation of EO on Sabouraud media with traps for keratinolytic fungi allowed us to isolate colonies of predominantly white fungi (shown by the arrow), which differed in appearance from typical dermatophyte colonies (Figure 2).

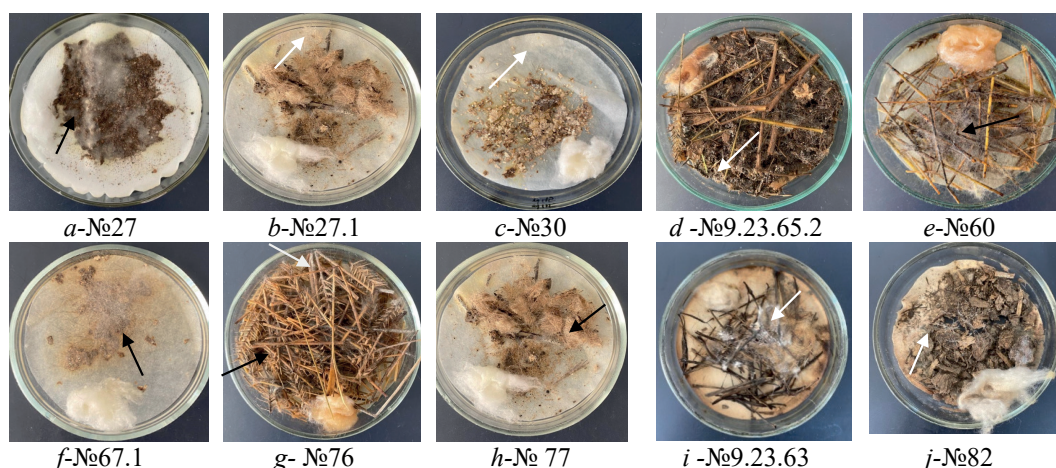


Figure 2 – Isolation of keratinophilic fungal isolates from soil (d, e, f, j), litter (a, b, c), feeders (g, h, i)

Figure 2 shows that in some Petri dishes, colonies of micromycetes developed on the hair. In samples № 27 (litter) and № 67.1 (soil), colony growth occurred along the length of the hair, and the formation of clearly visible white fluffy colonies was observed. In samples № 27.1 (soil) and № 60 (feeder), the formation of white colonies was noted along the entire hair and along the edge of the Petri dish. In sample № 30 (litter), the formation of dense colonies was observed, and in samples № 76, № 77 (scrapping from the soil) and in № 9.23.65.2, № 9.23.63, and № 82 - fluffy white colonies were observed. Analysis of all 113 strains of microorganisms isolated from samples of EO from the territory of livestock farms in four regions of Kazakhstan for the presence of keratinophilic properties showed that only 25 strains (22.1%) were positive for keratinase. It should be noted that of the total number of positive samples, keratinophilic bacteria (10 strains) were isolated in 40% of cases, and keratinophilic micromycetes (15 strains) were isolated in 60% of cases, respectively.

In all cases, keratinophilic bacterial strains were isolated from environmental samples collected from the Pavlodar region (Figure 3).

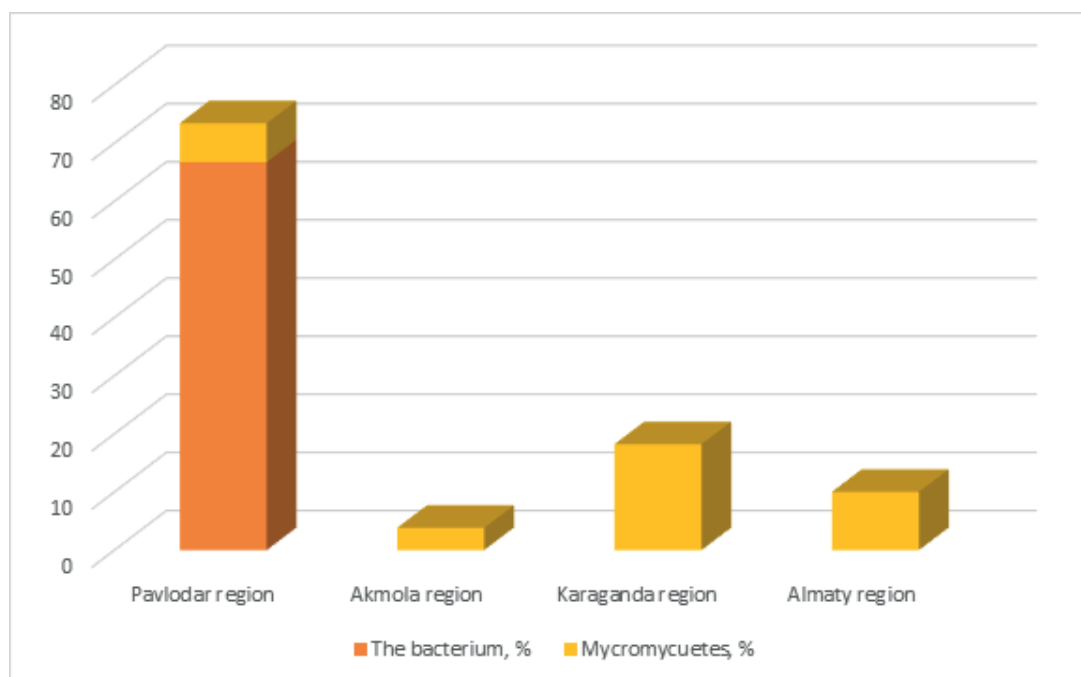


Figure 3 – Results of identifying keratinophilic strains of microorganisms from environmental objects by region of Kazakhstan

As shown in the diagram, by region, the percentage of keratinophilic fungal strains isolated from environmental samples collected in the Akmola, Karaganda, and Almaty regions was 3.8%, 18.2%, and 10.0%, respectively. In the Pavlodar region, bacteria (shown in green) were isolated in 66.7% of samples, which were not analyzed in further studies given the study's goal, and micromycetes were isolated in 6.7% of samples. It should be noted that a larger number of keratinophilic micromycetes were found in the samples of the Karaganda region's organic matter.

The spectrum of the isolated mycelial keratinophilic fungi consisted of representatives of the genera *Trichophyton* spp., *Phoma* spp., *Marquandomyces* spp., *Penicillium* spp., *Lecanicillium* spp., *Fusarium* spp., *Alternaria* spp., *Aspergillus* spp., *Filobasidium* spp., and *Mucor* spp. (Figure 4).

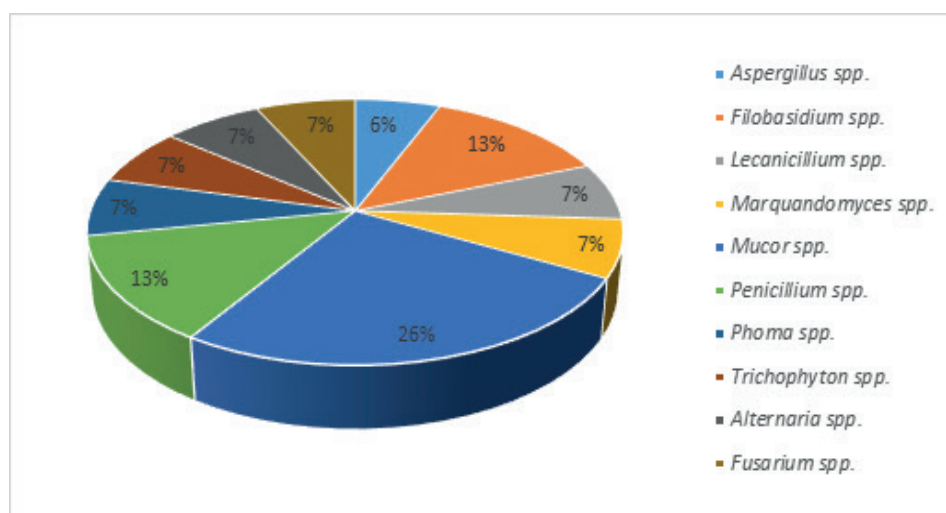


Figure 4 – Spectrum of mycelial keratinophilic fungi isolated from environmental objects

The identification of pure cultures of keratinophilic fungi isolated from the EOS by cultural and morphological properties allowed us to identify *Trichophyton spp.*, *Phoma spp.*, *Marquandomyces spp.*, *Penicillium spp.*, *Lecanicillium spp.*, *Fusarium spp.*, *Alternaria spp.*, *Aspergillus spp.*, *Filobasidium spp.*, *Mucor spp.*. The fungal cultures differed in terms of morphological features (Figure 5).

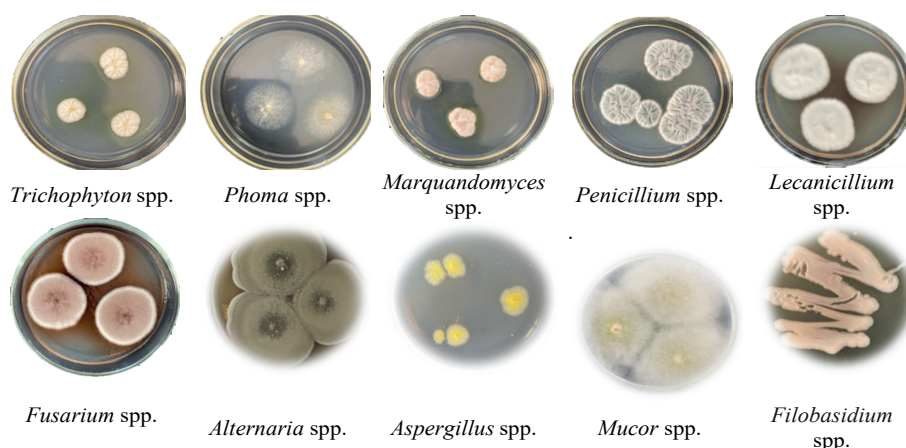


Figure 5 – Keratinophil cultures isolated from environmental objects

Figure 5 clearly shows the morphological features of the strains. The white *Trichophyton spp.* colony had a velvety surface, formed yellow exudate drops, and exhibited moderate growth. The *Phoma spp.* colonies had white fluffy mycelium on the front side, a light reverse, abundant yellowish exudate, and rapid growth. The *Marquandomyces spp.* isolate colony had a light cream, mealy surface with a translucent edge and dense consistency. The *Penicillium spp.* isolate formed classic, well-known, rounded and dense colonies of rich dark green color that are unevenly distributed over the surface of the substrate. The colony of the *Lecanicillium spp.* isolate had a dense, slightly fluffy texture and a snow-white color. The surface was velvety with clearly defined edges. The *Fusarium spp.* colony was light pink with white edges. It had a convex, folded surface and a crater-shaped center, and its edges were uneven. *Alternaria spp.* colonies were dark green, irregular in shape, with a bumpy profile and a rhizoid center. The edges of the colony were round and irregular, with mycelium in the form of threads. The isolated *Aspergillus spp.* formed colonies of a rounded shape with a bright yellow color, fluffy and velvety in texture, and with wavy, fuzzy edges. The isolated *Mucor spp.* colony was white with a cream center, fluffy, cotton wool-like, with fuzzy, blurred edges. The colony of the *Filobasidium spp.* isolate had a smooth, shiny, and dense texture of cream color. The microscopic identification results of the strains are presented in Figure 6.

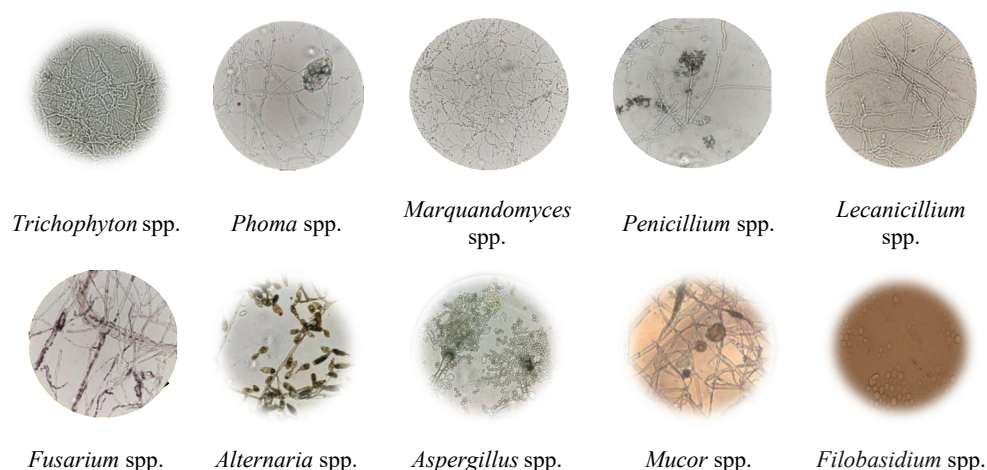


Figure – 6 Results of microscopic identification

As shown in Figure 6, *Trichophyton* spp. have thin septate hyphae, microconidia, and fusiform macroconidia. Upon microscopy, *Phoma* spp. exhibit the formation of pycnidia with unicellular ellipsoidal conidia. *Marquandomyces* spp. have separate hyphae. *Penicillium* spp. show tassel-shaped conidiophores and chains of spherical conidia. *Lecanicillium* spp. display branched conidiophores with phialides. *Fusarium* spp. have a septate mycelium of burgundy color. *Alternaria* spp. have septate, light brown mycelium and conidia with three to eight transverse and two longitudinal septa. *Aspergillus* spp. have septate hyphae and conidiophores with a bubble-shaped head covered with phialides and conidia. *Mucor* spp. form wide, aseptate hyphae with large sporangia. *Filobasidium* spp. are yeast-like fungi with thin, septate hyphae and blastospores. The results of the analysis for the presence of saccharolytic activity of some keratinophilic isolates are shown in Figure 7.

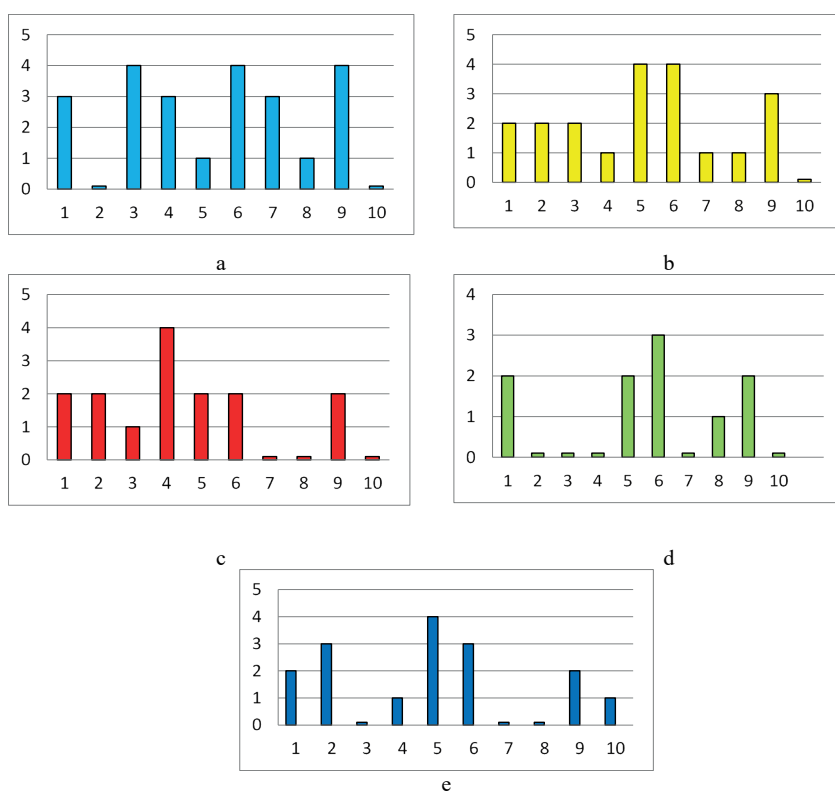


Figure 7 – The results of the saccharolytic activity of isolates:

1- *Trichophyton* spp., 2- *Phoma* spp., 3-*Marquandomyces* spp., 4-*Mucor* spp., 5-*Penicillium* spp., 6-*Lecanicillium* spp., 7- *Filobasidium* spp., 8- *Aspergillus* spp., 9- *Fusarium* spp., 10- *Alternaria* spp. isolated from environmental objects: a – sucrose, b – maltose, c – glucose, d – lactose, d – mannitol

According to Figure 7, sucrose was metabolized most actively by the isolates (85%), and maltose was well absorbed (77%). Glucose was absorbed in 69% of the isolates, mannitol in 62%, and lactose in 46% of the isolates. Thus, the isolates exhibited the greatest metabolic activity in relation to sucrose and maltose, which is probably due to their adaptation to these substrates in the environment.

The urease and proteolytic activity of the isolates was studied by assessing the cleavage ability of urea, gelatin, and casein on special media. The research results are shown in Figure 8.

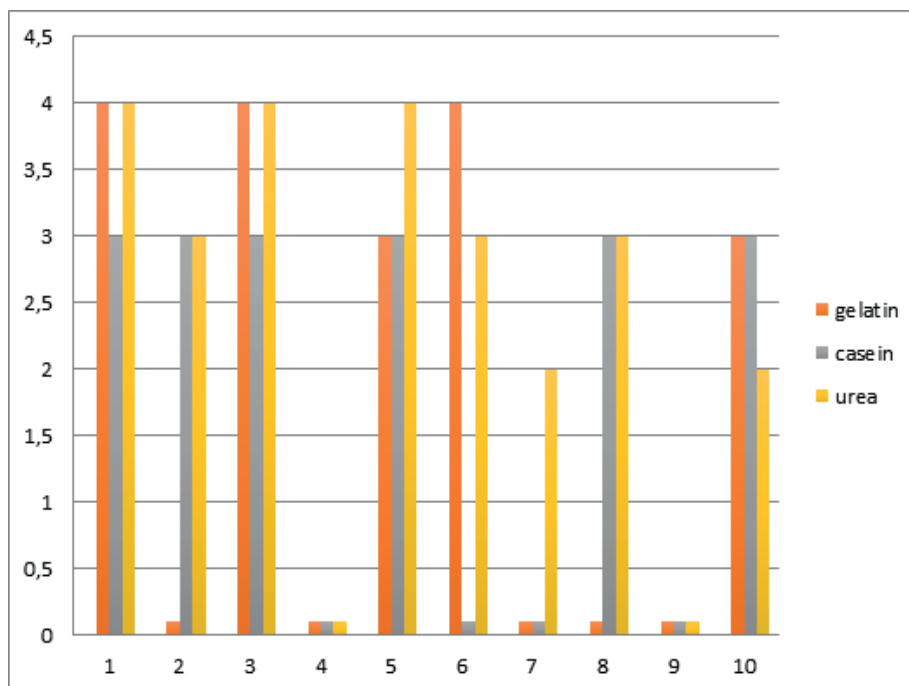


Figure 8 – Results of urease and proteolytic activity of isolates isolated from environmental objects

As shown in Figure 8, the largest number of isolates exhibited urease activity (70%), indicating their high metabolic activity against nitrogen-containing compounds. At the same time, hydrolytic activity against proteins (gelatin and casein) was noted in 50% of the isolates, indicating moderately pronounced proteolytic activity of the studied strains.

Thus, micromycetes exhibit a wide range of enzymatic capabilities, with greater activity toward urea.

The hair on which fungal growth was noted was examined under a microscope from each sample of EO to identify keratinophilic properties. The microscopy results are shown in Figure 9.

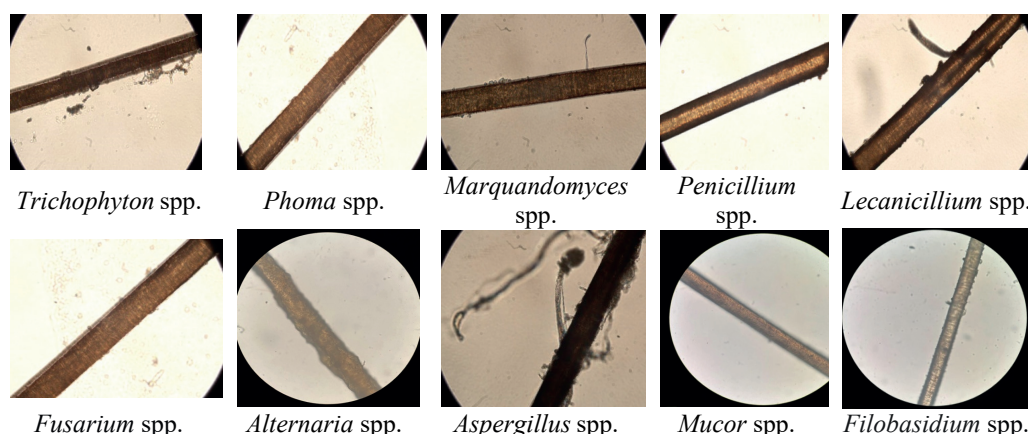


Figure 9 – Microscopy results of hair showing the growth of keratinophilic fungi:

a – *Trichophyton* spp., b – *Phoma* spp., c – *Marquandomyces* spp., d – *Penicillium* spp.,
e – *Lecanicillium* spp., f – *Fusarium* spp., g – *Alternaria* spp., h – *Aspergillus* spp., i – *Mucor* spp.,
j – *Filobasidium* spp.

As shown in Figure 9, *Trichophyton* spp. exhibited mycelium emerging from the hair. A small growth of fungal mycelium was observed in *Phoma* spp. *Marquandomyces* spp. had smooth hair and no visible colonies. *Penicillium* spp. showed destruction of hair with visible filaments, indicating an intensive decomposition process. *Lecanicillium* spp. had large filaments of fungal mycelium tightly surrounding the hair. *Fusarium* spp. appeared almost clean, but when magnified, individual spores were visible, indicating the presence of keratinophilic properties. The mycelium of *Alternaria* spp. was present around the hair. *Aspergillus* spp. showed good fungal growth around the hair, with a characteristic conidial head. *Mucor* spp. and *Filobasidium* spp. had smooth, even hair.

It is well known that the soil is a reservoir of geophilic keratinophilic fungi, whose biological role is to decompose keratin in the form of keratinized remnants of skin, its derivatives, and other possible sources of animal keratin in the soil, on the surface of the earth, and litter. The keratinophilic fungal species *Chrysosporium* spp., *Pseudogymnoascus* spp., *Geomyces* spp., *Pectinotrichum* spp., and *Renispora* spp. possess biologically significant properties, and a number of others can use predigested keratin residues or by-products of keratin degradation [34].

P. Shivanand, F.H. Yakop (2019) conducted a study on the mycobiota of the soils of forest ecosystems with a focus on geophilic fungi and their pathogenic potential. The studies were conducted on soil samples collected from various natural areas, including pine and mixed forests. Using molecular biological and cultural methods, genetically modified fungal species such as *Aspergillus* and *Penicillium* have been discovered, among which common pathogenic species such as *Aspergillus fumigatus* and *Penicillium marneffeii* [31, 35].

As part of the study, we analyzed 117 samples, of which the growth of micromycetes was recorded in 16 samples, representing 13.7% of the total number of samples. In the Akmola region, out of 26 species, only one appeared in one place (*A. cristatus*) for the first time (3.8%). In the Karaganda region, out of 66% of cases, it was noted in 10 cases (15.2%), where species such as *Lecanicillium saksenae*, *Penicillium chryseogenum*, *Filobasidium magnum*, *Mucor plumbeus*, *Trichophyton verrucosum*, *Phoma livicola* and *Marquandomyces* spp. were identified. In the Pavlodar region, out of 15 samples, growth was detected in one case (*Mucor plumbeus*), which is 6.7%. A similar situation was noted in the Almaty region, where, out of 10 samples, growth was detected in only one sample (*Mucor plumbeus*), representing a 10% growth rate. Samples in the amount of 101 (86.3%) micromycetes showed no growth, which is probably due to the lack of favorable conditions for micromycetes development. The main similarity between the previous studies and our results lies in the identification of the same dominant genera of fungi (*Aspergillus*, *Penicillium*).

The study by Z. Tyszkiewicz and M. Krasowska (2022) was devoted to studying the mycobiota of soils in buffer zones in the agricultural landscape in order to identify the diversity of micromycetes and their impact on carbon dioxide (CO₂) and nitrous oxide (N₂O) emissions. Soil samples were collected from two different zones: under trees near arable land and under grass cover near pastures. Several fungal genera have been identified using cultivation and microscopic analysis methods, including *Penicillium*, *Pseudogymnoascus* and *Chrysosporium*. *Pseudogymnoascus roseus* showed the highest amount in both soil types. We found that the soil under trees has a higher diversity of fungi as well as higher microbiological activity, which leads to increased emissions of CO₂ and NO₂. Both studies used cultural methods of fungal isolation and microscopic analysis and also considered the influence of soil characteristics on the diversity of micromycetes. A similar genus of fungi, such as *Penicillium*, was also identified in the works, which confirms their widespread distribution. However, unlike our study aimed at identifying keratinophilic fungi with pathogenic potential, the work of Tyszkiewicz and Krasowska focused on soil-based micromycetes in agricultural buffer zones and their impact on CO₂ and N₂O emissions. In addition, that study did not consider the pathogenic properties of fungi, whereas our analysis focused on the pathogens of opportunistic mycoses [36].

Various authors have reported the detection of fungi of various genera in the affected skin areas of domestic animals. Among them are the genera *Aspergillus*, *Alternaria*, *Chaetomium*, *Phoma*, *Penicillium*, *Cladosporium*, *Candida* and other representatives of mold fungi and yeasts [28, 29]. *Aspergillus*, *Penicillium*, *Fusarium*, *Microsporum*, *Trichoderma* and *Chrysosporium* are the most common cryptolytic fungi [13].

Our studies confirmed the isolation of representatives of fungal genera such as *Trichophyton* spp., *Phoma* spp., *Marquandomyces* spp., *Penicillium* spp., *Lecanicillium* spp., *Fusarium* spp., *Alternaria* spp., and

Aspergillus spp. from EO such as soil, litter from stalls, scrapings from feeders, and walls of livestock buildings, etc. spp., *Filobasidium* spp., *Mucor* spp. At the same time, a high frequency of isolation of keratinophilic *Aspergillus* and *Penicillium* species has been reported by many [31, 13]. Animals, in turn, are most often infected with fungal infections through contact with objects such as soil, water, and organic materials due to their high content of fungal spores.

Using the Hiss medium allowed us to evaluate the saccharolytic activity aimed at studying the ability of fungi to metabolize various carbohydrates, such as sucrose and maltose, which turned out to be the most digestible (85% and 77% of isolates, respectively), while lactose and mannitol demonstrated lower digestibility.

Proteolytic activity was evaluated to study the ability of fungi to break down proteins; 69% of isolates showed high activity on gelatin, but only 46% on milk, indicating their different enzymatic orientation. It should be noted that, along with keratinophilic properties, these representatives revealed the activity of other enzymes that promote the breakdown of various sugars and animal proteins: sucrose, maltose, mannitol, gelatin.

Urease activity was studied to determine the ability of fungi to decompose urea, which revealed positive results in 67% of the isolates, demonstrating their metabolic flexibility in conditions rich in nitrogen-containing compounds. The presence of high urease activity has been established, which indirectly indicates the potential to live on the skin of living organisms and assimilate nitrogen compounds, particularly urea. The data explain how fungi adapt to various substrates in the external environment, which are their natural reservoirs.

Based on the results obtained, we should agree with the opinion of scientists who stated that the ability of saprophytic fungi to destroy and assimilate keratin found in surface tissues places them in the category of opportunistic pathogens of skin mycoses and determines the pathogenicity of these fungi [10]. The widespread distribution of soil saprophytes with keratinophilic properties in the soil as a reservoir allows us to conclude that animals are infected not only with classical geophilic dermatophytes of the genus *Trichophyton* (for example, *Trichophyton ajelloi*, *Trichophyton flavescens*, *Trichophyton gloriae*, *Trichophyton terrestre*, *Trichophyton mentographytes*, variant gypseum, and a number of others), but also with opportunistic ecologically significant keratinophilic fungi.

Conclusion

Samples of EO (soil, litter from stalls, scrapings from feeders and walls of livestock buildings, etc.) were collected and revealed the presence of ecologically significant saprophytic keratinophilic micromycetes, including: *Trichophyton* spp. – 7%, *Phoma* spp. – 7%, *Marquandomyces* spp. – 7%, *Penicillium* spp. – 13%, *Lecanicillium* spp. – 7%, *Fusarium* spp. 7%, *Alternaria* spp. – 7%, *Aspergillus* spp. – 6%, *Filobasidium* spp. – 13%, *Mucor* spp. – 26%.

Among keratinophilic strains with high enzymatic activity, sucrose (85%), maltose (77%), glucose (69%), and mannitol (62%) were the most actively metabolized. Furthermore, 70% of the isolates exhibited urease activity, indicating high metabolic activity toward nitrogen-containing compounds. The activity of enzymes against proteins (gelatin and casein) was observed in 50% of the isolates.

All strains absorbed keratin to varying degrees. *Penicillium* spp. demonstrated clear hair destruction with visible filaments, indicating intensive keratin degradation. In contrast, *Trichophyton* spp., *Aspergillus* spp., and *Alternaria* spp. actively formed dense mycelium sheaths around the hair, but without pronounced structural damage, suggesting a strong colonization capacity rather than direct lysis of hair tissue.

Authors' Contributions

YK, TG and PR: Concept development, design and planning of the study, data collection and analysis, critical review of the article and final approval, research, statistical analysis. ZK and GB: Conducted a comprehensive literature search and conducting research. All the authors have read, reviewed and approved the final version of the manuscript.

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