Keratinophilic Fungi: Diversity And Abundance in the Soil of Ajmer District, Rajasthan

Renu Jangid[1,*], Shruti Ojha[1]

[1]Department of Botany, Maharshi Dayanand Saraswati University, Ajmer, Rajasthan, India.

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Corresponding author:

Renu Jangid, Department of Botany, Maharshi Dayanand Saraswati University, Ajmer, Rajasthan, India

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Abstract

Soil is a natural habitat and ecosystem for microorganisms, including bacteria and fungi. Mostly keratinophilic fungi are found in soil, use keratin as a growth substrate, and are essential to the natural degradation of keratin waste. These fungi include dermatophytes, a potential source of infectious diseases in humans and animals and cause dermatophytosis. One hundred thirty-six soil samples were collected from several sites including animal habitats in Ajmer district, Rajasthan, India. The soil samples were used for the study of keratinophilic fungi related to species richness, abundance, and diversity. The Physio-chemical properties of collected soil samples were analyzed and examined in soil microflora for temperature, pH, and macronutrients including nitrogen, phosphorus, and potassium. Ninety-eight soil samples (72%) showed positive results for the keratinophilic fungal isolates. The isolated fungal species belonging to eight genera and seventeen species included Aspergillus niger, A. flavus, A. fumigatus, Chrysosporium tropicum, Microsporum gypseum, M. canis, Fusarium solani, F. oxysporum, F. verticilloides, Trichophyton mentagrophytes, T. rubrum, T. tonsurans, T. terrestre, T. equinum, Penicillium, Mucor circinelloides, and Blastomyces. The temperature recorded for keratinophilic fungi ranged between 25°C and 37°C and pH was found in the range of 7.0 to 8.5. The fungal community was dominated by the order Onygenales followed by Eurotiales and Hypocreales belonging to the phylum Ascomycota.

Introduction

Soil is an important natural resource and habitat for many microorganisms. Fungi are the second largest population after bacteria in the soil. Fungi live as saprophytes in soil, and their occurrence in soil depends on the physicochemical parameters of the soil, which vary from place to place. The keratinophilic fungi grow on keratin residues present in soil [1, 2].

Keratinophilic fungi are the biggest fungal group that can break down keratin waste in nature. Keratin is a highly stable protein that is not easily degraded. The keratinophilic fungi secrete keratinase enzymes that degrade keratin residues in or at the soil [3, 4]. Keratinophilic fungi can be pathogenic or non-pathogenic. Several dominant keratinophilic fungi including Alternaria, Fusarium, Aspergillus, Geomyces, Chaetomium, and Penicillium have been isolated from arid to semi-arid environments such as New Mexico [5], Utah, USA [6], India [7], Bahrain [8],

Israel [9], and Chile [10]. The 'Hair-baiting technique' was initially developed by R Vanbreuseghem to isolate keratinophilic fungi from soil habitats [11]. Dey and Kakoti reported the first isolation of a keratinophilic fungal species, named Microsporum from soil in India [12, 13]. Labuda Roman et. al., (2024) reported two new species of K. kautmanovae sp. nov. and K. keniense sp. nov., which were isolated from soil samples collected from two distinct geographic and environmental sites (Africa and Europe)[14]. Many studies on the distribution of keratinophilic fungi have been carried out in various parts of the world [15-17].

Ajello (1953) reported two groups of fungi that occur in the soil, able to degrade keratin and live at the expense of keratinous substances. The first groups of fungi partially degrade keratin to utilize the protein, carbohydrates, and other keratin products. These fungi are referred to as keratinophilic fungi. The second group of fungi is colonized on the skin or dermis of humans and animals and is known as the dermatophytes [18]. Dermatophytes are classified on the basis of their habitat, nature and epidemiology into three groups such as Geophilic, zoophilic and anthropophilic. Geophilic fungi are saprobic, occur mainly in soil and are rarely pathogenic and have the ability to colonize keratinous substrates. Zoophilic fungi are mainly parasitic to lower animals and anthropophilic fungi are mainly parasitic on humans and theoretically evolved from geophilic dermatophytes. Skin infections are a serious health concern for people, particularly in tropical and subtropical regions like India where moisture is a key factor in promoting the growth of these fungi [19].

Keratinophilic fungi are important primarily for two major reasons. First, these fungi play a crucial role in ecosystem functioning and degrade three major portions of soil keratin waste, together with bacteria and actinomycetes, which otherwise would have been a major pollution problem. Second, these fungi are potential producers of industrially important enzymes such as keratinase which can be used to make creams, cosmetics, shampoos, hair conditioners, and pharmaceutical products. Nickerson (1947) suggested that the enzyme produced by keratinophilic fungi may act only on the reduced form of keratin [20].

The diversity of keratinophilic fungi and their diversity has been conducted across the globe from both humans and animals such as horsehair [21], feathers [22], human hair [23, 24], and human nails [25]. Despite the potential importance and prospects in the study of keratinophilic fungi, a huge research gap remains in the physio-chemical properties including soil pH, temperature, and soil nutrients. The abundance and diversity of this fungal community will provide important information regarding the seasonal and habitat preferences as well as suitable microenvironments in variable ecosystems. This research aimed to study the diversity, abundance, and physio-chemical properties of keratinophilic fungi in different habitats across the semi-arid ecosystem of Ajmer district, Rajasthan, India.

Material and Methods

Collection of soil samples

The soil samples were collected from selected locations of the Ajmer district, Rajasthan, India including S.P.C. Govt. College, Daulat Baagh, Mayo College, Gulab Bari, Madar, Pushkar, Panchsheel Nager, J.L.N Hospital, Beawar Road, Nasirabad, Anasagar Chowpati, Rasoolpura.

A total of one hundred thirty-six soil samples were collected during March-May and analyzed for further investigation. Soil temperature was checked at the time of sample collection by a digital thermometer. The collected soil samples were used for the analysis of physical and chemical parameters of the soil and isolation of Keratinophilic fungi.

Soil analysis

Physio-chemical properties of soil i.e., pH [26], temperature [27], electrical conductivity [28], and nutrients (Nitrogen [29], phosphorus [30], and potassium [31] were analysed.

Soil pH: The pH meter was calibrated with a buffer solution of 4 to 9.2 for soil pH analysis and checked with a glass electrode [25]

Electrical Conductivity

The conductivity meter was calibrated at room temperature with 0.01N Potassium chloride solution. Conductivity was checked for each sample using a conductivity cell by regular washing [26].

Nutrients NPK

Nitrogen content by alkaline permanganate, available Phosphorus by 0.5M NaHCO3, and available Potassium (K) by 1N NH4OAc extracts method was evaluated [27-29].

Isolation, purification, and identification of keratinophilic fungi

The hair-baiting technique of Vanbreuseghem (1952) was used to isolate keratinophilic fungi [10]. The Hair Baiting Technique is as follows: -

- The moist chamber was prepared using sterile Petri dishes and blotting papers.
- Half-filled the Sterile Petri dishes with collected soil samples.
- Spread 2-3cm short strands of sterilized defatted baits (hair, nail) over the soil surface.
- Sterile water was poured into the soil sample to facilitate the germination of fungal spores.
- Three replicate samples were processed.
- These dishes were incubated at room temperature (20-25oC) for 4 weeks.
- Pick one hair or nail with visible growth and culture it on Sabouraud's Dextrose Agar (SDA).
- Observe mycelium growth and colony.

A small section of fungal growth was picked up with the help of a sterilized needle and mounted on a slide. Under a light microscope, these isolated fungal growths were examined after being stained with Lactophenol Cotton Blue. Later, it was transferred to Sabouraud Dextrose Agar (SDA) with streptomycin (0.05mg/l) (SDA+A) in a glass petri dish (100 mm). For pure cultures, a mycelium plug was transferred to a fresh SDA+A at regular intervals of time to avoid contaminations. The culture petri dishes were incubated for 3-7 days at 27°C [32-35].

The microbiological techniques for the study of keratinophilic fungi, such as isolation and characterization, were used as per standard procedures using standard references including Description of medical fungi, and Pictorial atlas of soil and seed fungi [30, 31]. Dermatophytes gross and microscopic, and Laboratory methods in basic mycology [32, 33].

Statistical analysis

The correlation study of physico-chemical parameters was analyzed by multivariate analysis (The Principal component analysis method). A heatmap was constructed using PAST 4.03 statistics software for the evaluation of the relative abundance, diversity, and distribution of keratinophilic fungi across different habitats. The mathematical and descriptive analysis was conducted using Microsoft Excel 365 software. GraphPad Prism software 9.5.0 version was used for multivariate analysis. The diversity indi-

ces were analyzed for the study of various parameters including species richness/evenness, Shannon-Wiener Index (H'), Simpson's Similarity Index (SI), and species dominance using PAST 4.03 statistics software.

Results

Soil Analysis: - Soil samples were collected from twelve habitats including goat, cow, buffalo, dog, horse, duck, pigeon, crop field, college campus, roadside, garbage side, and public parks. The Physico-chemical properties of collected soil samples were analyzed and their data were represented in Figure 5.

The temperature recorded ranged between 250C and 370C. The pH was found in the range of 7.0 to 8.5. The highest conductivity was found in animal habitats and the lowest in road site soil samples. The highest amount of N, P, and K were observed in the duck habitat (205kg/na), pigeon habitat (12.05kg/na), and duck habitat (405kg/na). The lowest soil nutrients were observed in the horse habitat (142kg/na), the goat habitat (9.0kg/na), and the cow habitat (238kg/na) (Table-1).

Keratinophilic fungi

Out of the one hundred thirty-six collected soil samples, ninety-eight soil samples (72%) showed positive results for keratinophilic fungi Figure 1, 2. The majority of isolated species showed cutaneous mycoses in humans and animals. Twelve different habitats were studied for the isolation, abundance, and diversity of these fungi. Table-2, Figure 3 shows the percentage frequency of different fungal taxa belonging to different orders. These fungi found in soil samples taken from various habitats. The dog habitat reported the highest percentage (80%) of keratinophilic fungi in collected soil samples. In roadside soil, the percentage of keratinophilic fungi was 63%. Whereas, the duck habitat was recorded with a minimum distribution (50%) of keratinophilic fungi.

Diversity indices

In all the habitats studied, three sites including cow, buffalo, and dog showed the highest number of taxa and individuals whereas the lowest was observed from the duck habitat. The same three habitats showed species evenness of J=0.97, J=1, and J=0.9 respectively. However, contrary to individual and taxa numbers, duck habitat showed species evenness J= 1. For Shannon index diversity, Table 3 shows the highest range was shown by buffalo followed by cow and dog habitats with 2.39, 2.36, and 2.36 respectively. Similar to the previous diversity results, the duck habitat showed the lowest, with 1.09. However, contrary to the diversity indices results, species dominance was highest in duck (0.33), horse (0.25), and pigeon habitats (0.25) (Figure-6 (A), Table 3).

Distribution and abundance of keratinophilic fungi

The abundance and distribution of keratinophilic fungi are represented in the heat map Figure 4. The highest relative abundance in the heat map showed Trichophyton mentagrophytes from goat, cow, and dog habitats with two species each. The lowest relative abundance was observed from the duck habitat (total of 3 species) and the highest from cow and dog habitats (a total of 12 species each) (Figure 4). A total of eight genera and seventeen species of keratinophilic fungi were isolated across all habitats and areas. Following keratinophilic fungi were recorded predominantly: T. mentagrophytes, Aspergillus niger, Chrysosporium sp., Microsporum gypseum, Fusarium solani, T. rubrum, A. flavus, A. fumigatus, F. oxysporum, T. tonsurans, F. verticilloides, T. Terrestre, Penicillium, M. canis, Blastomyces (Table4, Figure 4).

T. mentagrophytes was isolated from the majority of samples, 13 samples with 13.26% in total. It was

Table 1. Physico-chemical parameters of collected soil samples from various habitats.

S. No.	Soil samples	pH	EC (ms)	N (kg/na)	P (kg/na)	K (kg/na)
1	Goat habitat	7.88	3.1	165	9.08	245.3
2	Cow habitat	7.35	0.25	168	9.88	238
3	Buffalo habitat	8.22	3.2	150	10.55	288
4	Dog habitat	7.92	0.3	157	11.12	311
5	Horse habitat	8.36	2.9	142	10.88	292
6	Duck habitat	7.11	0.14	205	10.05	405
7	Pigeon habitat	7.36	0.36	192	12.05	305
8	Crop fields	7.79	0.11	172	11.02	265
9	College campus	7.18	0.2	152	10.02	255
10	Public Park	7.9	0.14	167	11.3	312
11	Road site	7.65	0.1	188	11.65	284
12	Garbage area	7.55	0.23	180	9.81	280

Table 2. Percentage occurrence of keratinophilic fungi in various soil habitats.

S. No.	Soil Samples	No. of sample examined	No. of positive samples	% Occurrence
1	Goat habitat	12	9	75%
2	Cow habitat	16	12	75%
3	Buffalo habitat	14	11	79%
4	Dog habitat	15	12	80%
5	Horse habitat	6	4	67%
6	Duck habitat	6	3	50%
7	Pigeon habitat	6	4	67%
8	Crop fields	10	6	60%
9	College campus	12	9	75%
10	Public Park	9	7	78%
11	Road site	16	10	63%
12	Garbage area	14	11	79%

Table 3. Study the diversity of Keratinophilic fungi

	Habitat/Area												
	Goat	Cow	Buffalo	Dog	Horse	Duck	Pigeon	Goat	Crop fields	College Campus	Public Park	Road site	Garbage area
Taxa_S	7	11	11	11	4	3	4	7	5	8	7	10	10
Individuals	9	12	11	12	4	3	4	9	6	9	7	10	11
Dominance_D	0.16	0.09	0.09	0.09	0.25	0.33	0.25	0.16	0.22	0.13	0.14	0.1	0.1
Simpson_1-D	0.83	0.9	0.9	0.9	0.75	0.66	0.75	0.83	0.77	0.86	0.85	0.9	0.89
Shannon_H	1.88	2.36	2.39	2.36	1.38	1.09	1.38	1.88	1.56	2.04	1.94	2.3	2.27
Evenness_e^H/S	0.94	0.97	1	0.97	1	1	1	0.94	0.95	0.96	1	1	0.96

Table 4. Distribution of keratinophilic fungi in different soil habitats

	Habitat/Areas													
Keratinophilic Fungi	**Goat**	**Cow**	**Buffalo**	**Dog**	**Horse**	**Duck**	**Pigeon**	**Crop field**	**College campus**	**Public Park**	**Road side**	**Garbage site**	**Total**	**% Frequency**
Aspergillus niger	1	1	1	1	1	0	0	1	2	1	1	2	12	12.24%
Aspergillus fumigates	1	1	0	1	0	0	0	1	0	0	1	1	6	6.12%
Aspergillus flavus	1	1	1	0	0	0	0	1	1	1	0	0	6	6.12%
Blastomyces	0	1	0	0	0	0	0	0	0	0	0	0	1	1.20%
Microsporum canis	0	0	1	1	0	0	0	0	1	0	0	0	3	3.06%
Microsporum gypseum	1	1	1	1	1	1	1	0	1	1	1	0	10	10.20%
Trichophyton rubrum	0	1	1	1	1	0	1	0	1	0	1	1	8	8.16%
Trichophyton tonsurans	0	1	1	0	0	0	0	0	0	0	1	1	4	4.08%
Trichophyton terrestre	0	0	0	1	0	0	0	0	0	0	0	1	2	2.04%
Trichophyton equinum	0	0	0	1	0	0	0	0	0	0	0	0	1	1.20%
Trichophyton mentagrophytes	2	2	1	2	0	1	1	0	1	1	1	1	13	13.26%
Chrysosporium tropicun	1	1	1	1	1	1	1	0	1	1	1	1	11	11.22%
Fusarium solani	2	1	1	1	0	0	0	2	0	1	1	0	9	9.18%
Fusarium oxysporum	0	0	0	0	0	0	0	1	1	1	1	1	5	5.10%
Fusarium verticilloides	0	0	1	0	0	0	0	0	0	0	0	1	2	3.06%
Mucor circinelloides	0	0	1	1	0	0	0	0	0	0	0	1	3	3.06%
Penicillium chrysogenum	0	1	0	0	0	0	0	0	0	0	1	0	2	2.04%

more commonly recorded from slightly alkaline soil to a wide variety of habitats with a presence commonly in animal habitat sites. The second most commonly isolated species, Aspergillus niger was isolated from 12 samples (12.24%) from different habitats. The most commonly isolated species Chrysosporium tropicum was isolated from 11 soil samples with 11.22%. This fungus was isolated in the asexual state only. Microsporum gypseum was the fourth most prevalent geophilic fungal species in 10 samples with 10.20% from different habitats and more commonly recorded from slightly alkaline soil. Fusarium solani was the fifth most prevalent fungal species in 9 samples (9.18%). T. rubrum was the sixth most prevalent keratinolytic species isolated from 8 samples (8.16%) in different habitats. The remaining keratinophilic fungi isolated in the present study had a prevalence in the following descending order: A. flavus (6.12%), A. fumigatus 6 (6.12%), F. oxysporum 5 (5.10%), T. tonsurans 4 (4.08%), F. verticilloides 3 (3.06%), T. Terrestre 2 (2.04%), Penicillium 2 (2.04%), M. canis 2 (2.04%), Blastomyces 1 (1.02%) (Table 3). The abundance of Keratinophilic fungi also show in figure-6 (B).

Discussion

The present study focussed on a specialized fungal community known as keratinophilic fungi. The research emphasized understanding the abundance, habitat preference, diversity, and importance of the specific fungi.

Significance of soil analysis

Fungi play a crucial role in soil ecosystems, providing numerous benefits to plants and ecosystems. Soil analysis provides critical insights into fungal biodiversity and ecosystem health. Soil provides numerous benefits for Keratinophilic fungi, making it an ideal habitat for their growth and proliferation. Keratinophilic fungi thrive in slightly acidic to neutral soil [36, 37]. In this study pH of analyzed soil samples were found in the range of pH 7.0 to 8.5. Soil with abundant organic materials provides excellent conditions for fungal spreads. The amount of N, P, and K in soil affects the growth and sporulation of saprophytic fungi.

T. mentagrophytes and Aspergillus niger, the two most dominant taxa belonging to the order Onygenales and Eurotiales were found with high percentage frequency and abundant in different micro-environments of the selected locations (Figure 3). The environmental factors, including physico-chemical parameters of soil, play vital roles in the growth and prevalence of keratinophilic fungi. The varied prevalence of these fungi in soil of various habitats may be due to some differences in the climatic conditions [36].

M. gypseum was the most common geophilic fungus which depends on the occurrence of keratin source in different habitats and other factors viz., pH, temperature and inorganic substances also responsible for the occurrence of these fungi. Some geophilic species evolved in anthropophilic dermatophytes and cause infection in humans and animals. T. rubrum and T. tonsurans are both anthropophilic in nature, but in this study are found as geophilic. T. tonsurans was also isolated from unsterilised soil and SDA media at three different temperatures (room temp., culture room temp. and at 11ºC) conditions in Jaipur city, Rajasthan. The pathogenicity of these geophilic species is lower than zoophilic and anthropophilic dermatophytes but may rise in virulence when a host has low resistance to pathogens.

Keratinophilic fungi are considered dermatophytes due to their potential to degrade keratin and cause infection (dermatophytosis or mycoses) in humans and animals [38, 39]. Research shows the degradation of keratin present in soil proves to be an indicator of a high pathogenicity rate and related opportunistic pathogens [40]. The keratinophilic community in arid and semi-arid ecosystems could be

an important factor in increasing population susceptibility to infectious fungal pathogens.

Relative abundance, distribution, and diversity of keratinophilic fungi

In the present study, one hundred thirty-six soil samples were examined, and ninety-eight soil samples were found positive. A total of eight genera and seventeen species were isolated and identified. The dominant keratinophilic fungi observed were Trichophyton mentagrophytes, Aspergillus niger, Microsporum gypseum, Chrysosporium tropicum, and Fusarium solani. Kumawat et al. (2020) also reported the same genera of keratinophilic fungi. They isolated 154 isolates belonging to 31 keratinophilic fungi of 16 genera including Aspergillus terreus (4.19%), Fusarium solani (7.79%), Chrysosporium tropicum (11.04%), Chrysosporium indicum (5.84%), Microsporum canis (5.84%), Trichophyton mentagrophytes (8.44%) and Trichophyton rubrum (7.14%) [41]. Forty-seven samples were collected from animal and bird habitats and seventy-five isolates were isolated belonging to 14 genera and 20 species [42]. Order Onygenales being the most dominant are common in arid and semi-arid ecosystems. The fungi belonging to this order mainly require keratin for their growth. Moreover, this explains the reason these fungi show low culture growth in artificial growth media [43].Dermatophytic infections are increasing tremendously and have become the most common infectious disease in the world [6]. Studies show about one-fifth of the population of the world is suffering from mycotic infections [39].

Several studies show the distribution of keratinophilic fungi in various soil habitats across India and other countries. This explains the rich diversity and abundance of keratinophilic communities in the soil [44, 45]. The isolates evaluated in the present research have been reported from several other substrates and locations across the globe. Fungal isolates from desert grasses of Arizona and New Mexico [46, 47], and rhizosphere soil from west Iran [48] show high diversity and distribution of the fungi across varied ecosystems from desert to grassland and several others.

Conclusion

Soil is an ideal environment for the keratinophilic fungi. In the present study, the physico-chemical properties of soil and the isolation of keratinophilic fungi have been conducted. Human hairs were found best for the isolation of keratinophilic fungi. The occurrence of Trichophyton mentagrophytes (13.26%) was highest in soil samples followed by Aspergillus niger (12.24%). Keratin is the amplest of proteins on the earth. Bacteria and Keratinophilic fungi cycle keratin which is a highly stable protein in nature. The soil contains more species of keratinophilic fungi and other related dermatophytes than those presently recorded globally. A huge gap stands for further studies of keratinophilic fungi about their taxonomy and ecology.

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Disclosure Statement

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Author Contributions Statement

Renu Jangid structured the manuscript. Additionally, the laboratory experiments, data and concept design were also conducted by Renu Jangid. Shruti Ojha conducted statistical analysis, critical revision, and data interpretation of the manuscript.

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