

## A PRELIMINARY STUDY ON THE OCCURRENCE OF KERATINOPHILIC FUNGI IN REGIONAL PARKS OF KOTA, RAJASTHAN

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### ABSTRACT

Keratinophilic fungi are said to be a group of fungi that colonize various kind of keratinous substrates degrading them to components of low molecular weight. The aim of this study was to isolate and identify keratinophilic fungal flora from soil sample of Regional Park of Kota (Rajasthan). A total no. of 20 soil samples were screened out for the presence of Keratinophilic fungi by using hair baiting technique. These soil samples are rich in pathogenic Keratinophilic fungi including dermatophytes belongs to 6 genera and 7 species The Keratinophilic fungi isolates includes *Aspergillus niger*, *Aspergillus flavus*, *Penicillium species*, *Microsporum gypsum*, *Fusarium species*, *Trichophyton rubrum* and *Chrysosporium tropicum*. *Aspergillus niger* was found abundant in the isolates while the *Microsporum gypseum* was found to be the second prevalent fungi. This study will help in the future research related to the degradation of keratin which is abundant source of protein and very difficult to degrade. Hence to prevent the environment from such kind of products the microorganisms play very important role.

**Keywords-** *Keratinophilic fungi, Hair bait technique, Regional Park, Soil flora.*

### INTRODUCTION

Soil being a complex ecosystem is compound of several minute habitats and harbour almost all major taxonomic group of fungi along with bacteria and algae. Soil is rich in keratinous materials and most conducive medium for the growth and occurrence of Keratinophilic fungi. These fungi one of the nature's gifts which have ability to decompose even the hardest substance like keratin [1]. Keratinophilic fungi colonize different keratinous substrates and degrade them to components of low molecular weight. These fungi are natural colonizers of Keratin substrates and can be isolated by hair bait technique [2].

Keratinophilic fungi produce important enzyme keratinase that has proteolytic nature and play an important physiological function and also used to for industrial market purposes like production of citric acid and waste management and biotransformation [3]. The keratinophilic fungi in India were isolated from various habitats viz., public parks and soils or floor dust of primary schools [4]. lake side soils [5], birds and their environment [6] and saltpans [7]. Thus, in the present study the isolation and identification of keratinophilic fungi were performed from soil samples of the regional parks in Kota (Raj) for morphological analysis. Geographically the climate of Kota region is characterized by long and intense hot summer, The maximum temperature appearance of 45°C get recorded in the month of June, and the humidity also favour the growth of these kind of fungi So far, no study has been conducted for isolation of keratinophilic fungi from Kota city previously. Hence the present study reported the occurrence of keratinophilic fungi in this region.

## **MATERIAL AND METHODS**

### **Collection of Soil Samples**

In the study undertaken, a total no. of 20 soil samples were collected from 20 regional parks of Kota. Before collection of soil samples, superficial debris and other vegetative materials were removed from the soil samples. Loosened soil (approximately 500 g) samples were taken from the surface layer of each site at a depth of 2-5 cm. Soils were collected in sterile labelled polyethene bags by using spatula [8] and transferred to laboratory for further analysis.

### **Measurement of Soil pH**

The pH play important role in the growth and development of keratinophilic fungi, hence first parameters to be analysed was pH of each soil sample. It was measured after preparation of soil suspension (one gram of soil to 5 ml deionized water) using pH meter [9].

### **Isolation of Keratinophilic fungi**

Keratinophilic fungi were isolated by the hair baiting technique method in which human hair were used as keratin substrate. Soil samples were moistened with sterile distilled water

supplemented with antibiotic solution chloramphenicol (0.2g/ml) and transferred into sterile petri plates containing defatted humans' hairs and spread on to the soil plates [10]. These petri dishes were incubated at room temperature for 3-4 weeks in upright position. After observing colony morphology and growth these colonies were further subcultured on Sabouraud Dextrose Agar (SDA) medium supplement with antibiotic Chloramphenicol [11].

### Identification of Keratinophilic fungi

The fungi were identified by conventional method (colony morphology). Isolated fungi were then stained with lactophenol cotton blue and observed under the microscope to analyse the macro and microconidia characteristics [12].

### Tease mount preparation

A drop of Lactophenol Cotton Blue stain [LPCB] was placed on the non-greasy, sterile glass slide. With the help of the teasing needle a tuft of fungal hyphae/mycelium with the spores were placed on the slide and the strands of the hyphae were teased slowly and gently to avoid disruption of the conidia.

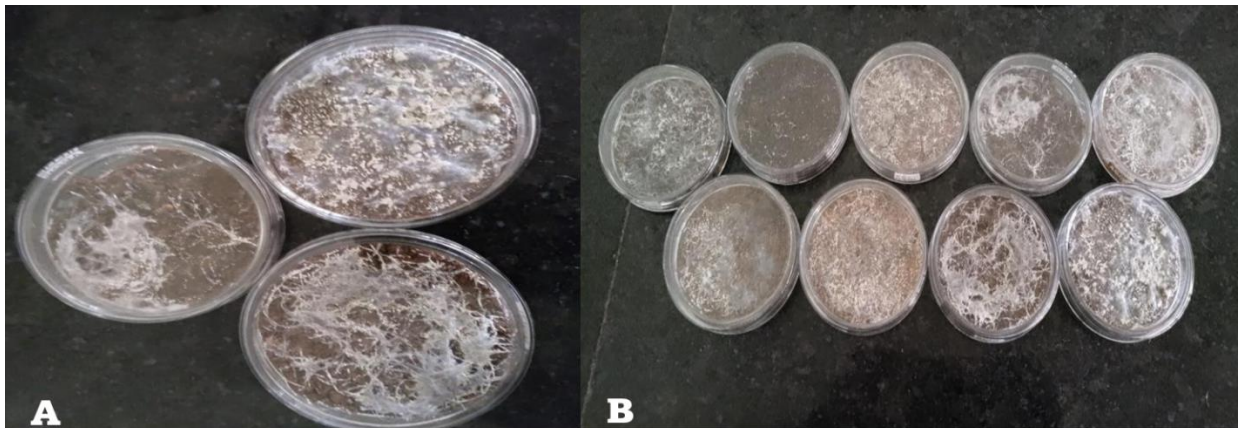
## RESULT AND DISCUSSION

A total 6 genera and 7 species of different keratinophilic fungi were isolated and classified from 20 soil samples obtained from various Regional Parks of Kota (Rajasthan). The isolated keratinophilic fungi characterized were of the following genera: *Aspergillus niger*, *Fusarium solani*, *Aspergillus flavus*, *Penicillium citrinum*, *Microsporium gypseum*, *Trychophyton rubrum*, *Chrysosporium tropicum* (Table-1 and Figure 1-2). Furthermore, the *Aspergillus niger* (Table-2) was found to be the most dominating (60%) fungi followed by the *Microsporium gypseum* (40%). Although other genera were found in low amount as compared to the dominating genera. Bentubo *et. al.*, [13] also reported that *Microsporium gypseum* as the most dominant dermatophyte in Brazil city. Some species of *Aspergillus niger* and *Chrysosporium tropicum* were found responsible to causes mycotic keratitis [14].

In the studies from several parts of India the fungal strains of *Aspergillus niger* and *Microsporium gypseum* were isolated in general as keratinophilic fungi and a few dermatophytes were also reported in the soil samples from several studies and reports [15].

Keratinophilic fungi produced are of great importance and play big role in bioremediation of pollutants in natural environment. A large number of keratinous wastes are generated every year mainly in the agricultural fields.

In previous studies *Microsporum gypseum* was reported as best keratinase producer and *Microsporum gypseum* was recognized as strong producer of extracellular enzyme keratinase in medium including porcine nail as the source of nitrogen and carbon [16]. Second most common species isolated in our study was *Microsporum gypseum*.



**Figure-1** Keratinophilic fungi were isolated from different soils samples A. using 'Hair bait technique' and B. growth of fungi after 15 days



**Figure-2** Isolated culture showing A. *Microsporum gypseum* and B. *Aspergillus niger*

Table – 1. Morphological and Microscopic characterization of fungal isolates

Fungal isolates	Morphological Characterization		Microscopically Observations
	Surface	Reverse	
<i>Aspergillus flavus</i>	Olive to lime green	Cream or pale	Mycelium septate, Conidial head are mostly radiate, conidiophores are rough, conidia are round with smooth to finely roughed walls, appear in chain
<i>Trichophyton rubrum</i>	White to bright yellowish beige or red violet	Pale or yellowish brown	Slender clavate to pyriform Microconidia are irregular shape, macroconidia are absent
<i>Aspergillus niger</i>	White to black	Pale yellow	Septate, unbranched mycelium, conidial head are mostly radiant, small rough conidia.
<i>Penicillium citrinium</i>	White to grey green	Pale, brown or deep yellow brown	Mycelium is septate and hyaline, smooth walled conidiophores stipes brush like cluster penicillin.
<i>Microsporium gypseum</i>	White to yellowish	Deep yellow or yellow orange	Septate hypha, Macroconidia are thin and rough contain 3to6 cells. Macroconidia are drop-shaped, pear and club shaped.
<i>Chrysosporium tropicum</i>	White cream tan to pale brown	White to brown	Septate hyphae, conidia are broad based, one celled and occur terminally on pedicels along the sides of hyphae.

Table - 2 Majority of fungal isolates obtained from various sample sites

S. No.	Fungal Isolates	Sample site (Regional Park)	% Isolation
1	<i>Aspergillus niger</i>	Dadabari, Kunhadi, Borkhera, Rangbari, Tipta, Nayapura, Gumanpura, Retwali, Keshavpura, Kishore Pura, Balabhbari R.K puram	60%
2	<i>Microsporium gypseum</i>	Kunhadi, Sabarmati Colony, Tathed, Mahaveer Nagar, Talwandi, Gumanpura, Vivekananda Nagar, Kishorepura	40%
3	<i>Aspergillus flavus</i>	Rajiv Gandhi Nagar, Dadabari, Talwandi Balabhbari	20%
4	<i>Trichopyton rubrum</i>	Sabarmati Colony, station	10%
5	<i>Candida albicans</i>	Mahaveer Nagar, Nayapura	10%
6	<i>Penicillium citrinum</i>	Tipta. Vivekananda Nagar	10%
7	<i>Chrysosporium tropicum</i>	Station	5%

Moorthy K *et. al.*, in 2011 found similar results as our study reported through his research and found *Aspergillus niger* (60%) as most dominating fungi followed by *Aspergillus flavus* (20%) among the isolates obtained from pigeon feathers and also, he found the presence of other organisms including *Absidia* spp. *Acremonium* spp. *Aspergillus fumigatus*, *A. glaucus*, *A. ochraceous*, *Chrysosporium* spp. and *Trichoderma* spp. to be 10%. His experiments on Rabbit feathers led him to the isolation of *Aspergillus niger* and *Alternaria* spp. Moorthy K *et. al.*, also stated that *Fusarium* spp. and *Rhizopus* spp. were equally occurred in cow hairs (20%). *Aspergillus niger* was the abundant fungal isolate (70%) occurred in goat hairs followed by *A. flavus*, *A. glaucus*, *A. ochraceous* and *Cladosporium* spp. which were recorded as low as only 10%. He also studies these fungal isolates in cat and dog hair samples equally [17].

## CONCLUSION

Keratin is one of the most abundant and important animal proteins on earth as it plays important role as building block part of the exoskeleton of reptiles, birds, and mammals. Among the microbes to manage the cycle of this protein in nature, keratinophilic fungi are very common and the most diverse. During evolution, many soil-associated fungi belonging to the group of keratinophilic fungi adopted a life cycle which is pathogenic and known to be the most potential agents of fungal diseases in humans and animals. If keratinophilic fungi were absent or failed to cycle the highly stable protein (keratin), the quantity of keratin would rise beyond imagination and would accumulate on earth, since a vast quantity of Keratin is shed by the vertebrates. Indian soils contain more varieties of keratinophilic fungi than those presently recorded and there is need for further taxonomic and ecological studies of this interesting group of these kinds of organisms. The keratinophilic fungi produces keratinase enzyme which is very useful in the degradation of keratin and associated substances. These enzymes play important role in the food industries, waste management, leather industries and detergent industries. Beside these the keratinase is also important to silk degumming and medical fields.

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