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A Preliminary Study on the Occurrence of Keratinophilic Fungi in Soils of Bhandup Pumping Station

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ABSTRACT

The soil on Earth is infused with multiple crucial elements one of which is saprophytes which decompose organic matter. Keratinophilic fungi are one such saprophyte which has the ability to decompose even the hardest substance like keratin, which is otherwise resistant to degradation by most of the other microorganisms. Through biodegradation, they colonise in various keratinous substrates, produce enzyme keratinases, and decompose them into components with lower molecular weight. The present study was carried out with the aim of identifying keratinophilic fungi from the soil samples collected at Bhandup Pumping Station. Its unique location and geographic diversity makes it a potential area to study the distribution of keratinophilic fungi. The isolation technique used is the keratin bait technique in which the baits used were feathers of different birds collected from the study area. A total of four species were isolated from the culture which are *Aspergillus spp., Rhizopus spp., Trichophyton spp., and Fusarium spp.* The pathogenic keratinolytic fungal species produce extracellular keratinases which have applications in various industries. So, keratinophilic fungi might be pathogenic with regard to keratinized tissues of humans and animals.

Key words*: Keratin, Keratinophilic fungi, Keratin bait technique, Biodegradation, Saprophyte*

Introduction

Nature has provided planet Earth with a variety of beneficial organisms. The soil on Earth is infused with multiple crucial elements one of which is saprophytes which decompose organic matter. Keratinophilic fungi found in soil are one such ecologically important group of saprophytes which have the ability to decompose even the hardest substance like keratin and utilise it as the sole source of carbon and nitrogen (Sharma and Rajak, 2003). Keratin is the most abundant and highly stable animal protein present on the earth, and it is otherwise resistant to degradation by most of the other microorganisms (Sharma and Rajak, 2003). Keratins are divided into two types; α-Keratin in the form of a folder chain, present in wool, hair, and horn and β-Keratin in the form of a polypeptide chain, present in feathers (Kannahi and Ancy, 2012). Soil provides a heterogeneous and complex environment for all types of soil inhabitants. Soil is a good source of Keratinophilic fungi and the probability of the incidence of such fungi increases manifold if it is rich in keratinous materials (Sharma and Choudhary, 2015). The prevalence of these fungi depends on different factors, such as the presence of keratin sources, soil pH, temperature, and geographical location (Ashwathanarayana and Naika, 2016).

With this background in mind, the present study was carried out with the aim of isolating keratino-

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philic fungi from the soil of the Bhandup Pumping Station. Its unique location and geographic diversity make it a potentially interesting area to study the distribution of keratinophilic fungi which are responsible for the degradation of keratin. Keratinophilic fungi are important groups of fungi living in the soil that colonise various keratinous substrates and secrete many useful enzymes.

The biological function of keratinolytic fungi in the soil is the degradation of keratinized materials such as hides, furs, claws, nails and horns of dead animals (Sharma, R., & Rajak, R. C. 2003). The present study investigated a number of soil samples for the presence of Keratinophilic fungi and is the first study to be carried out in Bhandup at Bhandup Pumping Station.

Materials and Methods

Area of Study: Bhandup Pumping Station (BPS) is situated along Thane Creek, which is located partly on the coastline of the Mumbai metropolis (19° 07' 30" N,72° 57' 30" E). The area was selected as has a diverse habitat that includes mangroves, salt pans, and scrublands, which is the breeding as well as a nesting site for a large number of avian species. Bhandup Pumping Station and adjoining areas were surveyed for the collection of soil samples and feathers of different birds. It was an ideal location with fewer disturbances for sample collection. Address: - O-1, Eastern Express Highway, Tata Colony, Mulund East, Mumbai- 400081.

Collection of samples

Six soil samples and bird feathers were collected from different areas of Bhandup Pumping Station for the isolation of keratinophilic fungi. The soil samples were collected from a depth not exceeding 3-5 cm. Soil samples were collected and brought to the laboratory with sterile equipment such as forceps, spatula, and zip-lock bags for further processing.

Processing of soil samples

The 'hair baiting' technique initially developed by R Vanbreuseghem, a Belgian mycologist in 1952 was undertaken (Sharma and Rajak, 2003). Potato Dextrose Agar (PDA) was selected as the culture media. It was prepared and sterilised for 15 minutes in an autoclave at 394.15 K. The antibiotic streptomycin was added, and the media was poured into the petri dishes and left for solidification. The feathers were cut into small pieces and surface sterilised using Sodium Hypochlorite solution for two minutes. The process of culturing and mounting of fungal growth was done strictly under the Laminar Air Flow Machine (LAM) to avoid any type of contamination in the culture and glass slides. A total of 15 petri plates were made by adding different feathers and soil samples collected from different locations in multiple combinations on each plate. The plates were then incubated at room temperature for 2-3 days until the appearance of fungal growth. Macroscopic and microscopic examinations of the fungal growth were made with the help of standard procedures.

Identification of isolates

The mounting of fungal growth is a crucial process in the identification of fungal species. A drop of Lactophenol Blue stain [LCB] was placed on the sterile glass slide. With the help of the needle and forceps, a tuft of fungal hyphae/mycelium with the spores was placed on the slide. The slides were then observed under low power [10X] and high-power [45X] objective lenses. The colony morphology of the grown fungal colonies was observed by the pigment production in both the front and reverse sides of the plates.

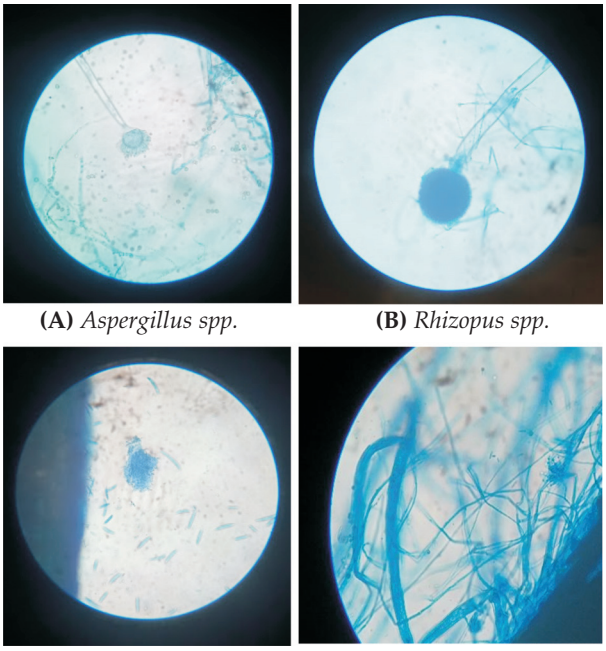
Results and Discussion

The results of the occurrence of keratinophilic fungi in the soil samples collected from Bhandup Pumping Station show the prevalence of keratinophilic fungi. All the soil samples collected were positive

Table A. Contains fungi observed and identified during the study.

Sr. No.	Division	Order	Genus	No. of species noted
1)	Ascomycota	Eurotiales	*Aspergillus.*	*1*
2)	Mucoromycota	Mucorales	*Rhizopus*	*1*
3)	Ascomycota	Hypocreales	*Fusarium*	*1*
4)	Ascomycota	Onygenales	*Trichophyton*	*1*

for fungal growth. The data reveals that the most frequently observed fungi were *Aspergillus spp., Rhizopus spp., Trichophyton spp. and Fusarium spp.* Among the four fungal species, *Aspergillus spp.* was found to be the first and most common fungi from all the soil samples, followed by *Rhizopus spp.* Hyphal entrance in bird feathers caused the disruption of the feather. It was observed that tufts of mycelia attacked the feather rachis from which the conidiophores of *Aspergillus spp.* and *Rhizopus spp.* developed. The degradation activity of *Aspergillus* was observed on feathers. *Trichophyton spp.* showed features such as fungal filaments detached from the strand surface and also covering the feather strand. *Fusarium spp.* is a potential keratinolytic fungal species, acting effectively on keratin substrates. Generally, keratin degradation by filamentous fungi occurs first by producing hyphae that grow around the keratin strand.



(A) *Aspergillus spp.* **(B)** *Rhizopus spp.*

(C) *Fusarium spp.* (D) *Trichophyton spp.*

Table B. Shows images of **(A)** *Aspergillus spp.,* **(B)** *Rhizopus spp.,* **(C)** *Fusarium spp. and* **(D)** *Trichophyton spp.* under a compound light microscope.

Potential features in the recycling of keratin protein are discovered by the process of keratin degradation from these fungi. The pathogenic keratinolytic fungal species produce extracellular keratinases which have many applications in industries such as leather industry, detergent industry, textile industry and degradation of feather waste (Kumar *et al.,* 2021). In the leather industry, the dehairing process of skin and hides requires keratinolytic activity which can be done using keratinophilic fungal enzymes.

They can be used as major pollution controllers in the leather industry. The utilisation of keratinases derived from these fungi can be used as a supplementary component in detergents for eliminating blockages in drains by degrading keratinous material such as hair, nail, etc. These fungi are also useful in Textile industries as they are capable of degrading dye, which is otherwise resistant to biological degradation. The presence of keratinophilic fungi enhances the breakdown of feather waste through the production of keratinase enzymes, leading to an increase in soil nutrient quality and hence can be used in fertilisers.

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Conflict of Interest: The authors declare no conict of interest.

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