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Keratin-Baiting Technique: Method For Isolation Of The Keratinophilic Fungi From Soil

Renu Jangid[1], Shruti Ojha[2]

[1,2]Department of Botany, Maharshi Dayanand Saraswati University, Ajmer, Rajasthan, India

Abstract: Keratinophilic fungi are ecologically important eukaryotic organisms that are known to play a diverse role in ecosystem stability and functioning. Some Keratinophilic fungi are pathogenic and have the ability to invade keratinized tissues of human being and animals to produce infection, dermatomycosis. Keratinophilic fungi produce the keratinase enzyme which degrade the most stable protein known as keratin on the earth. Keratin is a long, strong, strand-like protein that are insoluble in water naturally present in hair, nails, feathers, wool, hooves and hides. In nature millions of tons of keratin waste discarded from the poultry, meat, leather and textile industries that can lead environment pollution. Over the last decades, many researchers had reported and isolated keratinophilic fungi. These specialized keratin-degrading fungi adapt to varied environmental conditions such as soil and other dermatophytic substrates. These fungal group degrade keratin waste which can used for purposes such as nutrition in animal feed or fertilizers, medicines and cosmetics. The hair baiting technique is known to culture and isolate keratinophilic fungi in a much simpler and economically cheaper way. This article summarizes the hair baiting technique given by Vanbreuseghem and modified keratin-baiting technique to isolate and identify the fungi known for keratin degradation. Additionally, the important steps and ecological role of keratinophilic fungi in soil have also been discussed. It is believed that this specialized functional group of fungi will play a major role in ecosystem stability, functioning, and recycling for the future resources. This review also gives new shape to a special field of study medical mycology with various skin infections.

Key-Words **-** Dermatomycosis, Ecological role, Keratinophilic fungi, Keratin-baiting technique.

I. INTRODUCTION

The Keratin Baiting Technique is a specialized method to isolate keratinophilic fungi from soil samples. This technique involves dermatologic substances including hair, nails, and feathers for bait substrate to isolate keratinophilic fungi from soil (Hamada et al., 2024). Keratinophilic fungi are one of the largest groups of fungi capable of degrading keratin residues. Keratin is a highly stable protein on earth that is not easily degraded. Keratinophilic fungi utilize keratin through enzymatic digestion as a nutrient substrate for growth. This is often confirmed through visual observation of degradation and biochemical assays to identify keratinase enzymes responsible for keratinolytic activity (Sharma & Rajak, 2003; Simpanya & Baxter, 1996). The growth of keratinophilic fungi is reported to be slower in comparison to other fungal species on normal growth media. Concerning other growth media including DS-18 media and potato dextrose agar media, mycosel agar media (MYC) is known to isolate keratinophilic fungi at a higher rate than other growth media (Dolenc-Voljč, 2016; Hamada et al., 2024).

The keratin (hair) baiting technique was first reported by Vanbreuseghem in the early 20th century as a selective method for isolating keratinophilic fungi from soil (Vanbreuseghem, 1952). He proposed a hypothesis related to the saprophytic life of dermatophytes and observed that dermatophytes and keratinophilic fungi have been found in all five continents. To improve its efficiency and reduce contamination risks, this procedure has undergone various modifications. With the help of this technique

keratinophilic fungi from different places of the world have been reported by many researchers (Ajello, 1953, 1974; Battelli et al., 1978; Khan et al., 2020; Pakshir et al., 2013)

In India, the first report of keratinophilic fungi isolation was *Microsporum* from the soil by Dey and Kakoti. Keratinophilic fungi can degrade keratin, which is part of man and animals (Dey & Kakoti, 1955). Keratinophilic fungi have been receiving the considerable attention of mycologists and dermatologists all over the world because of their association with human and animal mycoses (Kumar et al., 2012; Lee et al., 2011). The last two decades has observed evolution of these fungi for the processes of keratin degradation (Sharma et al., 2011).

Keratin baits are used as nutrients source for the growth of keratinophilic fungi. Keratin baits are derived from different kind of keratin-rich substrates found abundantly in nature. The primary sources of keratin baits include: Hair, nails, feathers, animal wool, and other sources. The use of keratin baits is crucial for isolating and studying keratinophilic fungi from soil samples. The effectiveness of each bait varies depending on the specific fungal species being studied (Kotwal & Sumbali, 2016; Sharma & Rajak, 2003). Studies indicate that feather baiting is more successful than hair baiting in trapping these fungi, which may be due to better nutrient availability in feathers (Kotwal & Sumbali, 2016).

II. KERATIN-BAITING TECHNIQUE

The keratin-baiting technique includes basic steps:

1. **Sample collection**: Soil samples are collected from various locations and habitats, such as roadside, parks, hospitals, agricultural fields, etc. using sterile tools to avoid contamination. Dermatophytic samples including hair, nails, hair, and other substances are also important sources of isolation.
2. **Preparation of soil samples**: The collected soil is spread evenly on sterile Petri dishes.
3. **Inoculation with baits**: Sterile baits are placed on the surface of the soil which serves as a source of keratin for the fungi.
4. **Moistening and incubation**: The soil is moistened with sterile distilled water and incubated at room temperature (20-27°C) in the dark for 4-6 weeks to promote fungal growth.
5. **Observation and isolation**: After incubation, any fungal colonies that develop around the bait are sub-cultured on appropriate growth media (e.g., Sabouraud Dextrose Agar, Mycosel agar media). Fungal colonies are further subjected to identification and characterization (Table-1, Figure-1).

Table-1. Globally isolated keratinophilic fungi by baiting technique.

Keratin bait	Isolated Fungi	Location	Year	Reference
Hair	*Scopulariopsis, Chrysosporium*, *Arthrographis, Fusarium*	Osaka City, Japan	2024	(Hamada et al., 2024)
Hair	*Penicillium, Fusarium, Arthroderma, Keratinophyton, Nannizzia, Paraphyton*	Southwestern Poland	2024	(Spychała et al., 2024)
Hair	*Aspergillus, Trichophyton, Microsporum, Rhizopus, Pencillium, Chrysosporium, Fusarium.*	Rajasthan, India	2023	(Sharma et al., 2023)
Feathers	*Aspergillus, Chrysosporium, Fusarium, Chaetomium, Emericella., Papulospora,*	Iraq	2023	(Al-Bader, 2023)

	Cunninghamella, Rhodotorula, Acremonium, Cladosporium etc.			
Feathers	*Copulariopsis, Chrysosporium, Trichophyton, Microsporum, Myriodontium, Aphanoascus fulvescens*	Tuscany (central Italy)	2021	(Nardoni & Mancianti, 2021)
Hair and Nail	*Chaetomium, Emericella, Cochliobolus, Penicillium, Alternaria, Aureobasidium*	Saudi Arabia	2018	(Alwakeel, 2018)
Hair and Nail	*Chrysosporium,Microsporum, Trichophyton, Paecilomyces, Acremonium*	Iran	2012	(Kachuei et al., 2012)
Hair	*Acremonium, Aphanoascus, Arthroderma, Chrysosporium, Ctenomyces, Gymnoascus, Microsporum* etc.	U.P., India	2009	(Singh et al., 2009)
Hair	*Microsporum, Chrysosporium, Keratinophyton, Trichophyton,*	Malaysia	1991	(Soon, 1991)
Hair and Nail	*Chrysosporium, Trichophyton, Acremonium, Aspergillus, Penicillium. Chaetomium, Cladosporium* etc.	Egypt	1990	(Abdel-Hafez et al., 1990)
Hair	*Aphanoascus, Chrysosporium, Gymnoascoideus, Microsporum, Nannizzia, Pseudoarachniotus, Pseudoarachniotus*	Orissa, India	1980	(Sur & Ghosh, 1980)
Hair	*Microsporum, Arthroderma, Ctenomyces, Chrysosporium*	Pavia, Italy	1975	(Caretta & Piontelli, 1975)
Wool	*Gliocladium, Paecilomyces, Trichophyton, Diheterospora, Fusarium*	India	1966	(Simpanya & Baxter, 1996)
Hair	*Keratinophyton, Microsporum, Nannizzia, Trichophyton, Chrysosporium*	India	1965	(Randhawa & Sandhu, 1965)
Hair	*Microsporum gypseum*	United state	1953	(Ajello, 1953)

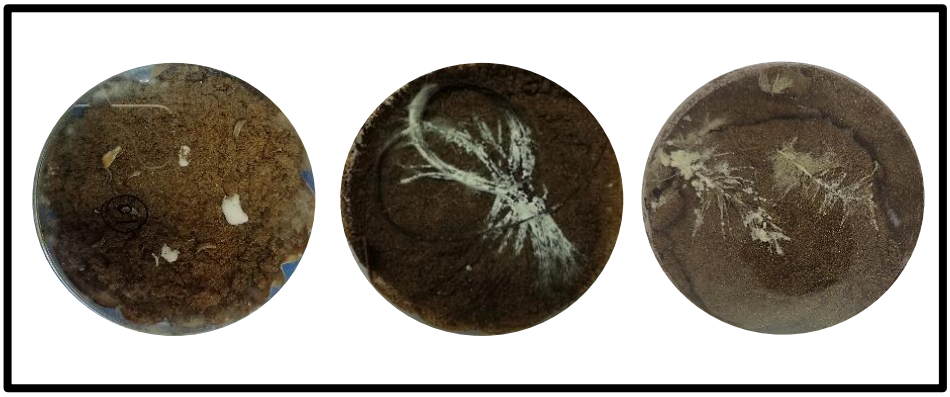

Figure 2. Keratinophilic fungi isolation by baiting technique. A. Nails. B. Hair. C. Feather.

III. APPLICATIONS OF KERATIN-BAITING TECHNIQUE

Keratinophilic fungi play a crucial role in ecosystem functioning and degrade the major portion of keratin waste together with bacteria and actinomycetes, which otherwise would have been a major pollution problem. Some keratinophilic fungal species that can cause skin infections in humans and animals (Malek et al., 2013; Pakshir et al., 2013). Keratinophilic fungi are produced keratinase enzyme by degrading keratin residues. This enzyme will play important roles in agricultural and environmental chemistry due to its ability to degrade keratins from various sources (Li et al., 2022). Keratin is produced in large quantity as a byproduct from poultry, slaughterhouse, tanning, and fur processing industries. With developing urbanization, these industries produce millions of tons of keratin waste. Few organisms are known to degrade keratin and utilize it due to its enzymatic undigested nature and stability. Therefore, their disposal leads to environmental problems such as air, soil, and water pollution. Every year more than 20,000 tons of feathers are produced as waste by poultry farming. Keratin is a major component of feathers. Keratinophilic fungi can degrade keratin, so feathers do not accumulate in nature (Kunert, 1989; Malviya et al., 1992; Suman Bahuguna & Kushwaha, 1989). Keratinase enzyme also used for purposes such as nutrition in animal feed or fertilizers, medicines, and cosmetics. The technique can help to yield a variety of fungal species, and understanding the distribution and prevalence of these fungi in different soil types can provide insights into their ecological roles and potential health risks (Hamm et al., 2020; Moorthy et al., 2016) (Figure-2).

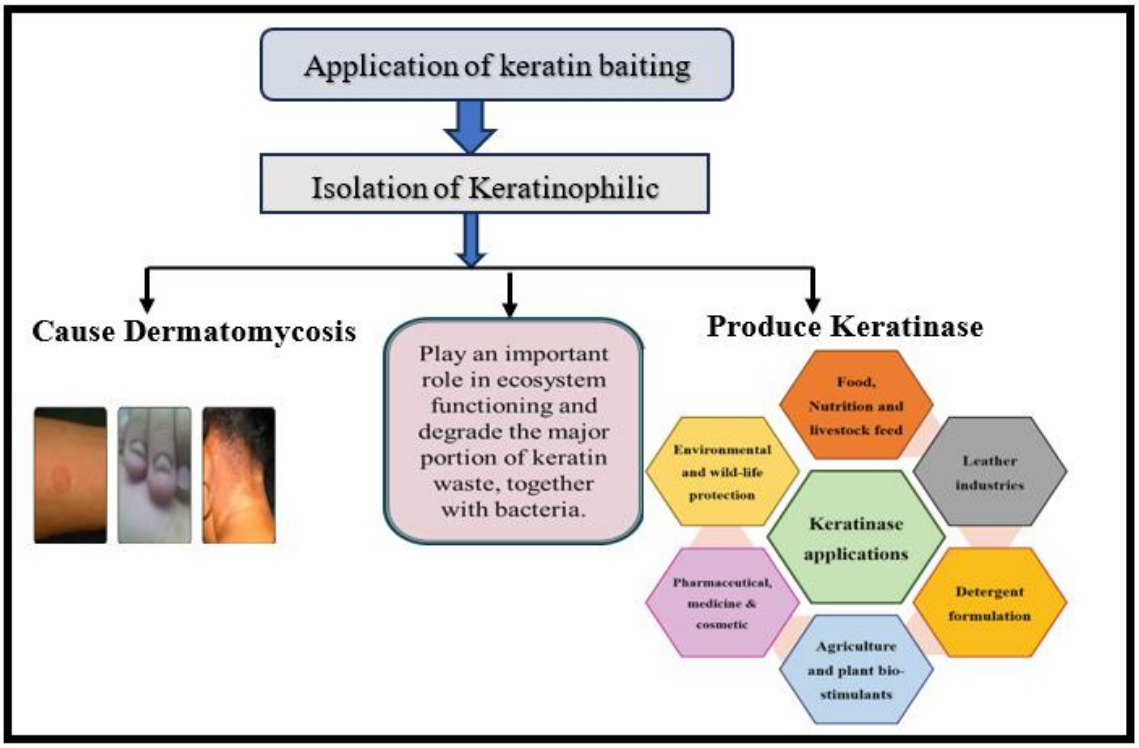

Figure 2. Applications of Keratin-Baiting technique.

IV. CONCLUSION

The keratin-baiting technique is a valuable method for isolating keratinophilic fungi from soil, contributing to our understanding of these fungi, their ecological roles, and their potential impacts on public health. Through careful execution of this technique, researchers can effectively identify and study various fungal species that thrive in diverse environments. This technique remains a fundamental method in mycology for isolating keratinophilic microorganisms and performs an important role in the sustainable management of keratin waste.

V. CONFLICTS OF INTEREST

The authors have no conflicts of interest.

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