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Microbial diversity of Keratinophilic Fungi –An excellent means of degrading keratinous waste, from Taj Mahal Agra, India

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Abstract-

Purpose :- Keratin waste from various sources like leather industry, poultry farms, slaughterhouses hold a major threat to environment and is one of the major contributors of environmental pollution. According to USA Foreign Agricultural services the large consumption of chicken meat is generating alarming amount of chicken feathers which are keratinous waste material. The Keratinophilic microflora is a significant component of soil and possesses the ability to degrade the highly stable animal protein on earth. These microorganisms utilize keratin by enzymatic digestion as a source of nutrient substrate for growth. The present study was conducted to isolate keratinophilic fungi from the surroundings of Taj Mahal, Agra, India.

Method: - In the present investigation, 67 soil samples were analyzed for keratinophilic fungi isolated from the surroundings of Taj soil Agra, India. Eleven different genera of keratinophilic fungi were isolated. The studies continued up to 12 months, from the year 2017-2018. These studies were concentrated upon various keratinous substrates such as feathers, human hair, horn and hooves.

Result:- Chrysosporium was the most dominant, followed by Trichophyton, Microsporum, Pectinotrichum, Neocosmospora, Zygonema, respectively. For the growth of keratinophilic

fungi on different substrates, feather was excellent and most of the fungi occurred on feather. Chrysosporium queenslandicum (MTCC 3333) (strain 2) showed maximum percent prevalence (71.4%) on feather, followed by C. tropicum (MTCC 3195) (60%), C. keratinophilum (MTCC 1367) (66.6%), T. simii (ATCC 16448) (57.1%), Geomyces pannorum (ATCC 34151) (57.1%), C. pseudomerdarium (57.1%), T. phasilioformae (57.1%).Chrysosporium keratinophilum and C. queenslandicum (strain 2) showed maximum prevalence (61.5% and 63.2% respectively) on hair. On horn, the maximum percentage prevalence (52%) was shown by C. keratinophilum. On hooves (30%) Penicillium chrysogenum (MTCC 3321) was observed.

Conclusion:- The soil which is a major source of keratinophilic fungi were collected in such a way that the succession of whole year can be taken into account. The appearance and disappearance of the fungi at the same place will give the variety of keratinophilic fungi and lead to complete degradation of the keratin which may be of various types like feather, hair, horn & hooves. It is relevant to state that the diversity of the fungi during the succession will be different at different spots (as mentioned in the four parks of Taj).

Keywords: Keratinous waste, Keratinophilic fungi, Agra soil, feather bait technique, Taj Mahal

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Introduction

The top producers of keratin waste are the United States of America, China, India, and Brazil. They manufacture keratin in the millions of tonnes. According to a recent estimate, India alone contributes 350 million tonnes of protein. 2010 (Agrahari and Wadhwa). Microbiological diversity is an important component of the biosphere. Its research and good use are important considerations when discussing biodiversity. There is an immense range of environment across the wide Indian subcontinent, which is reflected in the diversity of indigenous fungal flora.

The biodiversity concentrations of keratinophilic fungi were examined during a 12-month period (2017-2018) from Taj gardens in this study.
Humans, animals, and birds are all susceptible to these dermatophytes and keratinophilic fungi. Human hair, wool, and peacock feathers are consumed by some plant pathogenic dematiaceous fungus (Evans and Hose 1975), saprophytic fungi (Safranek and Goos 1982), and geophilic fungi (Kushwaha 1983; Nigam and Kushwaha 1992). According to Gupta and Nayak (2015), the potential of geophilic dermatophytes to destroy diverse keratinous surfaces has not been fully researched (feather, hair, leather). Both geophilic dermatophytes and other soil keratinolytics, on the other hand, could be pathogens. This can be confirmed by consulting the Atlas of Clinical Fungi (Hoog & Guarro 1995). Keratinophilic fungus do not rely only on keratin for their nitrogen supply. They are common in soil and can be identified with keratinous bait. Several Trichophyton species

According to Sharma et al., it had the maximum deterioration of animal hair (49.34 percent) (2011). Previous research on keratinophilic fungi focused on their geological distribution rather than their mycological characteristics. The prevalence of these fungi is substantially related to the health of humans and animals (Griffin 1960; Pugh 1980; Harison et al 2009). Other ecological factors influence the frequency of existence of geophilic dermatophytes and keratinophilic fungi, according to several researchers (Dey and Kakoti 1955; Garg 1966; Otcenasek 1978; Nigam and Kushwaha 1989, 1990; Saidi et al 1994). Some researchers discovered keratinophilic fungus in various soil environments surrounding Agra, but not in the

Taj location (Singh and Kushwaha 2010; Saxena et al 2004). According to the most recent information from the Uttar Pradesh government,

Materials and Method

The Taj Mahal city of Agra is located at an altitude of 766 metres above sea level and receives 60-100 cm of annual rainfall. The climate is semi-arid, with winter temperatures ranging from 2-3°C to 10-15°C and summer temperatures ranging from 25-27°C to 40-47°C. In sterile polythene bags, soil samples were gathered from areas of the Taj garden that were heavily disturbed by human activity. The bagged samples were stored at room temperature with the soil moist until dry, using a sterilised scraping hook. Pebbles, grass, seed traces, and other debris were sieved out of each sample. Peeled samples were taken from the upper layer of soil, around 5-8 cm deep. At room temperature, the soil samples were stored.

Results

For the isolation of keratinophilic fungus, two sampling areas from the Taj garden were chosen. The dominating genus was discovered to be Chrysosporium. Trichophyton, Microsporum, and Pectinotrichum, for example, were less common. Spots A and B in Park 1 were sampled, and the soil was cultured with four different types of keratin (feather, human hair, horn and hooves). Chrysosporium keratinophilum (MTCC 1367), Chrysosporium keratinophilum strain 1, Chrysosporium tropicum, and Trichophyton simii (ATCC 16448) were found on feathers, whereas all of the above fungi, as well as Chrysosporium keratinophilum strain 2, strain 3, were found on hair. Chrysosporium keratinophilum and strains 1, 2 were discovered on horns. Spot A was not aided by hooves. Geomyces pannorum (ATCC 34151), Microsporum canis, and Trichophyton megninii were discovered at park 1 Spot B.

Chrysosporium carmichaelii (GPCK 597), a Chrysosporium anamorph of Arthroderma curreyi, was found on feathers and human hair in park 2 spot E, as well as Penicillum chrysogenum (MTCC 3321) on hairs, horns, and hooves. In comparison to horn and hooves, the majority of the fungus were found on feathers and hair. Trichophyton rubrum (MTCC 296) and Trichophyton phasilioformae were isolated from feathers at spot F, but both of the above fungi were abundant on human hair, with the exception of Neocosmospora sp, which was also detected on horn and hooves.

Spot C Chrysosporium pseudomerdarium strains 1, 2, and 3 were discovered on feathers and hair in park 3. Only strains 1 and 2 were found on horns, while no fungus was found on hooves. On feathers, Chrysosporium pseudomerdarium strain 2 had the highest prevalence (75%), whereas strain 1 had the lowest (55%). (Table 2). Trichophyton rubrum, Trichophyton phasilioformae, and Chrysosporium anamorph of Arthroderma curreyi were identified on feather and hair, with highest frequency of 50 percent, 60 percent, and 57.1 percent and 44 percent, 55 percent, and 51.2 percent, respectively. Neocosmospora sp. (MTCC 3319) was only found on 40 percent of hairs, 37 percent of horns, and 29 percent of hooves, respectively.

Chrysosporium indicum was most common on feathers in park 4, spot G. (60 percent) Except

for Chrysosporium synchronum, which was discovered on 40% of feathers and 60% of hair, Pectinotrichum illiase was found on both feathers and hair. There was no fungal development on the horns or hooves. Chrysosporium queenslandicum (MTCC 3333) (strains 1 & 2) were identified on feathers with the highest prevalence (60 percent and 71.4 percent, respectively) and were isolated from hair (43 percent and 63.2 percent). Zygonema dermatitidis and Myceliophthora sp were only discovered on hair in 60 percent and 33.3 percent of cases, respectively. Fusarium letritium (MTCC 3320) was found at a maximum prevalence of 40 percent on horns and 21.3 percent on hooves.

Table I. Dermatophytes and related keratinophilic fungi associated on different substrates from four park sites of Taj, Agra, India.

Site	Spot	Feather	Hair	Horn	Hooves
Park 1	Spot A	4,5,16,29	4,5,6,7,16,29	4,6,7	-
	Spot B	18,19,25	18,19,20,25	25	19,25
Park 2	Spot E	1,2	1,2,24	24	24
	Spot F	27,28	22, 27, 28	22	22
Park 3	Spot C	8,9,10,11	9,10,11	9,10	-
	Spot D	14, 20	14,18	27,28	28
Park 4	Spot G	3,15,23,26	3,23,26	-	-
	Spot H	12,13,14	13,14,21,30	17	17

1. *Chrysosporium* anamorph of *Arthoderma curreyi* Berk
2. *Chrysosporium carmichaelii* Van-oorchot
3. *Chrysosporium indicum* (Randhawa & Sandhu) Garg
4. *Chrysosporium keratinophilum* D. Frey ex Charmichael
5. *Chrysosporium keratinophilum* Strain 1 D.frey ex Charmichael
6. *Chrysosporium keratinophilum* Strain 2 D.frey ex Charmichael
7. *Chrysosporium keratinophilum* Strain 3 D.frey ex Charmichael
8. *Chrysosporium pseudomerdarium* Van-oorchot
9. *Chrysosporium pseudomerdarium* Strain 1 Van-oorschot
10. *Chrysosporium pseudomerdarium* Strain 2 Van-oorschot
11. *Chrysosporium pseudomerdarium* Strain 3 Van-oorschot
12. *Chrysosporium queenslandicum* Apinis & Rees
13. *Chrysosporium queenslandicum* Strain 1 Apinis & Rees
14. *Chrysosporium queenslandicum* Strain 2 Apinis & Rees
15. *Chrysosporium synchronum* Van-oorschot
16. *Chrysosporium tropicum* Charmichael
17. *Fusarium letritium* Sheldon
18. *Geomyces pannorum* Sigler & Charmichael
19. *Microsporum canis* Hasagawa et al.
20. *Mucor pusillus* Lindt
21. *Myceliophthora* species Van- oorschot
22. *Neocosmospora* species Smith
23. *Pectinotrichum illiase* Varsavsky & Orr
24. *Penicillium chrysogenum* Thom
25. *Trichophyton megninii* Blanchard

26. *Trichophyton mentagrophytes* Smith & Marpales
27. *Trichophyton phasilioformae* Borelli & Feo
28. *Trichophyton rubrum* Castellani
29. *Trichophyton simii* Syockdale, Mackenzie & Austwick
30. *Zygonema dermatitidis* (Gilchrist & Stokes) Dodge

Table II: Percentage Occurrences of Fungi on Different Substrates

S.No	Name of Isolates	Feather (%)	Hair (%)	Horns (%)	Hooves (%)
1	*Chrysosporium* anamorph of *Arthoderma curreyi* Berk	50	44	0	0
2	*Chrysosporium carmichaelii* Van-oorchot	60	42	0	0
3	*Chrysosporium indicum* (Randhawa & Sandhu) Garg	60	46	0	0
4	*Chrysosporium keratinophilum* D. Frey ex Charmichael	66.6	61.5	52	0
5	*Chrysosporium keratinophilum* Strain 1 D.frey ex Charmichael	40	35	0	0
6	*Chrysosporium keratinophilum* Strain 2 D.frey ex Charmichael	40	33	0	0
7	*Chrysosporium keratinophilum* Strain 3 D.frey ex	60	49	0	0
8	*Chrysosporium pseudomerdarium* Van-oorchot	57.1	50	0	0
9	*Chrysosporium pseudomerdarium* Strain 1 Van-oorschot	60	55	46	0
10	*Chrysosporium pseudomerdarium* Strain 2 Van-oorschot	75	51.3	0	0
11	*Chrysosporium pseudomerdarium* Strain 3 Van-oorschot	40	33	26	0
12	*Chrysosporium queenslandicum* Apinis & Rees	40	0	0	0
13	*Chrysosporium queenslandicum* Strain 1	60	43	0	0

	Apinis & Rees				
14	*Chrysosporium queenslandicum* Strain 2 Apinis & Rees	71.4	63.2	0	0
15	*Chrysosporium synchronum* Van-oorschot	40	0	0	0
16	*Chrysosporium tropicum* Charmichael	56	56	0	0
17	*Fusarium letritium* Sheldon	0	0	40	21.3
18	*Geomyces pannorum* Sigler & Charmichael	57.1	42.3	0	0
19	*Microsporum canis* Hasagawa et al.	42.6	36.2	23	0
20	*Mucor pusillus* Lindt	0	66.6	0	0
21	*Myceliophthora* species Van- oorschot	0	33.3	0	0
22	*Neocosmospora* species Smith	0	40	37	20
23	*Pectinotrichum illiase* Varsavsky & Orr	60	54.3	0	0
24	*Penicillium chrysogenum* Thom	0	40	32	30
25	*Trichophyton megninii* Blanchard	50	48	35	21
26	*Trichophyton mentagrophytes* Smith & Marpales	33.3	29.1	0	0
27	*Trichophyton phasilioformae* Borelli & Feo	57.1	51.2	0	0
28	*Trichophyton rubrum* Castellani	60	55	0	0
29	*Trichophyton simii* Syockdale, Mackenzie & Austwick	57.1	48.3	0	0
30	*Zygonema dermatitidis* (Gilchrist & Stokes) Dodge	0	60	0	0

Discussion

Four sampling locations were chosen for the survey of keratinophilic fungi isolated from the Taj Garden in Agra. All of the spots yielded sixteen Chrysosporium spp. Feather was discovered to be the most beneficial of all the substrates.

Keratinophilic fungi are generally mesophilic in nature, and they are also a source of nutrients for plants and animals, primarily nitrogen and sulphur (Kornillowicz-Kowalska and Bohacz 2011), though some species, such as Chrysosporium keratinophilum, Chrysosporium tropicum, and Chrysosporium queenslandicum (Garg et al. 1985; Kumawa (MTCC 3333). However, most geophilic dermatophytes thrive at temperatures between 25 and 30 degrees Celsius.

For most keratinophilic fungi, the ideal temperature range is 25-27°C, with no growth over 40°C. Keratinophilic fungi are mostly mesophilic, according to a few findings, however some strains are thermotolerant and can adapt to extreme temperatures for survival (Pursola and Guarro 1984). They can degrade keratinous waste by producing keratinase enzyme, and soil is their preferred habitat because it contains both keratinous and organic material that can serve as a substrate (Sharma et al. 2015a,b).
Keratinophilic fungus can be found in a variety of settings where animals and humans congregate, including playgrounds, recreation centres, poultry farms, cattle pans, stables, zoological parks, swimming pools, and other places where animals and men congregate. Various types of keratin were utilised in the culture to assess their potential as keratinophilic fungus substrate (Lange et al 2016). Microsporum canis, Trichophyton rubrum, and Trichophyton mentagrophytes survived on skin scrapings, according to Knudsen (1980). Cornely et al. (2001) found Neocosmospora vesinfecta to be harmful in a patient with non-lymphocytic leukaemia in Germany (Garg et al 1985). The patient was infected in Nigeria, and symptoms began to develop on day 14 of the infection. Chemotherapy and large dosages of Amphotericin B, an antimycotic medication.

Fusarium moliniformis was found in 22% of poultry bird feathers (Cornley et al 2001). In the Galapagos Islands, Chrysosporium indicum, Chrysosporium keratinophilum, and Chrysosporium tropicum have been found in disintegrating lava soils with minimal organic matter (Sinski T et al 1987). Fusarium species were found in 0.47 percent of 236 samples taken from parks, school grounds, paddocks, river, roadside, and other locations (Kaul and Sumbali 2000). Six soil samples were collected along the Ross Sea coast, comprising a variety of G. pannorum isolates from Trichophyton mentagrophytes (Ajello and Padhya 1974)

Microsporum canis (8.1 percent), Chrysosporium keratinophilum (40 percent), and Penicillium (24.4 percent) were found on the baits in a study on keratinophilic fungi from rice fields, demonstrating the adaptability of these fungi in soil and their capacity to colonise any substrate provided to them (Simpanya and Baxter 1996). Thus, despite variations in climatic conditions and continual human meddling during agricultural methods, the incidence of this specific group of fungus in Indian soils has been demonstrated as a stable population with a relatively high incidence. The major genera Chrysosporium (13.4%) and Penicillium (14.5%) were found near the Chilka Lake in Orissa (Mercantini et al 1993).

The soil samples tested from the chicken farm had a pH range of 7.29 to 8.44, according to Godheja and Shekhar (2014). Chrysosporium species dominated the geophilic mycoflora isolated, with Chrysosporium tropicum accounting for 33.3 percent of the total. The correlation of all species isolated from skin scrapings and soil was significant at (P 0.05 and P 0.01 respectively) in statistical analysis. According to Saidi et al., Chrysosporium is the most common genus, with Chrysosporium tropicum being the most prominent species (Sunderam 1987).

Although several studies have been conducted to determine the long and short term survival of certain pathogenic keratinophilic fungi in fresh water and sewage habitats (Bohacz J 2017; Deshmukh SK 2014), the bottom sediment area is often neglected, and while there are other studies in fresh water and sewage environments, few studies have been conducted to determine the long and short term survival of certain pathogenic keratinophilic fungi in fresh water and sewage habitats (Cook and Schlitzer 1981). Keratinophilic fungi have been found in bottom sediments, according to RKS Kushwaha (2014).

Because it is closer to the main stairs of the monument, where every tourist passes to see the Taj, Park 1 and specifically location A revealed the highest quantity of mushrooms on the hair in this study. On feathers, Chrysosporium queenslandicum (strain 2) had the highest prevalence. The pathogenic Neocosmospora sp. was originally discovered in this location. Neocosmospora sp. was identified from hair bait, with a prevalence rate of 40%, 37% on horns, and 20% on hooves, respectively. Keratinophilic fungi were limited to a few common Chrysosporium, Malbranchia, and Myceliophthora species in this setting.

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