

Isolation and Identification of Keratinophilic Fungi from Burning Ghat Soil of Jharkhand Region of India

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ABSTRACT

Forty eight soil samples were collected from 12 burningghat soils of Ranchi, Jharkhand and screened for presence of keratinophilic fungi using hair baiting techniques for isolation. Fungal growths appearing on the baits after two to four weeks of incubation at room temperature were microscopically examined and cultured on mycological media. Cultures were then identified on the basis of colonial and microscopic features. A total of 107 colonies of different keratinophilic fungi were isolated from 48 soil samples. The isolated keratinophilic fungi were classified into 9 species belonging to 6 genera. The isolated keratinophilic fungi were in the following order of dominance: *Rhizopus stolonifer* and *Aspergillus niger* (14.02%), *Mucor puillus* (13.08%), *Chrysosporium* sp. (12.15%), *F. Oxysporum* (11.2%), *A. Terreus* and *Penicillium chrysogenum* (9.34%) and *A. Flavus* and *Penicillium* sp. (8.41%).

Key Words: Burning Ghat, Hair baiting technique, Keratinophilic fungi.

INTRODUCTION

Keratinophilic fungi are small, well defined and important group of fungi that colonize various keratinous substrates and degrade them to components of low molecular weight. These fungi are present in the environment with variable distribution patterns which depend on factors, such as human and or animal presence. Keratinophilic fungi represent an important component of soilmicroflora where they decompose the highly resistant keratin. Frequency of occurrenceof keratinophilic fungi in the soil was influenced by a number of biotic and abiotic factors (Otsenasek, 1978).The species of keratinophilic fungi have been divided into three categories depending on their natural habitats: anthrophilic, when human beings are natural host, zoophilic, when animals act as natural host and geophilic, when they inhabit soil.Reports on the presence of these fungi in different soil habitats from different countries e.g. Egypt (All and El-Sharouny, 1987), Australia (Rose, 1980), Palestine (Ali-Shtayeh, 1989), Spain (Clavo et al., 1984), India (Pandey et al., 1989; Anbu et al., 2004; Ganaie et al., 2010; Deshmukh et al., 2010), Korea (Lee et al., 2011), Iran (Mahmoudabadi et al.,

2008), Kuwait (Al-Musallam, 1989), and Malaysia (Soon, 1991) have shown the fact of its worldwidedistribution. Some of the keratinophilic fungi are known to be dermatophytes which cause superficial cutaneous infections (dermatophytoses) of keratinized tissues (skin, hair and nails) of humans and animals. There are reports on the presence of dermatophytes and other keratinophilic fungi from different habitats of India (Singh et al., 1994; Ramesh and Hilda, 1999; Deshmukh, 1999, 2002, 2004;Deshmukh et al., 2006, 2010). However there are no reports on keratinophilic fungi in Jharkhand, India. The present investigation was therefore undertaken to record the natural occurrence of keratinophilic fungi in the burning ghat soils of Jharkhand state of India.

Materials and Methods

Collection of soil samples and baits:

Forty eight soil samples were collected randomly from 12 garbage dumping sites (4 samples per site) from the Ranchi district of Jharkhand. Before collection of soil samples, superficial debris and other vegetative materials were removed from the soil surface. Loosened soil (approximately 500g) were

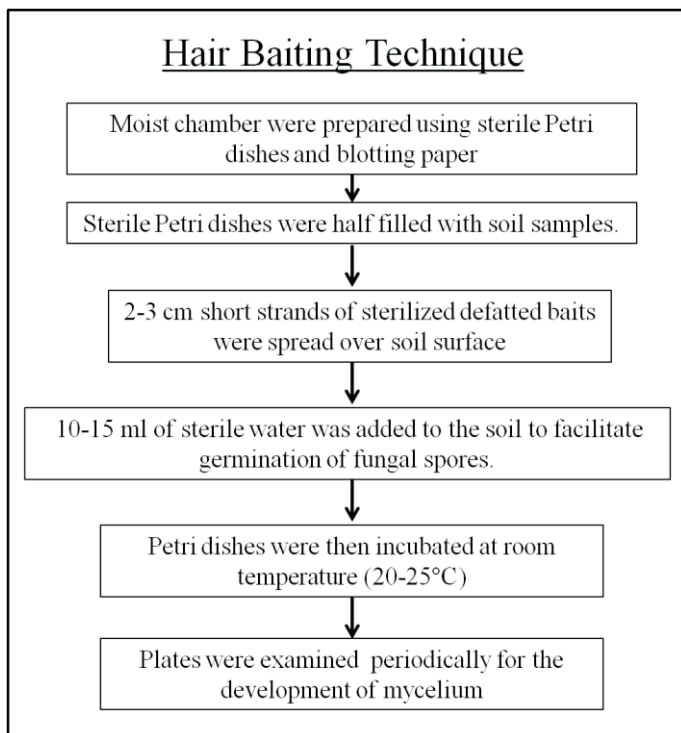
taken from the surface layer of each site to a depth of 2-5cm. Soils were collected in sterile plastic bags and sealed on the spot. Samples were brought to the laboratory and used immediately or stored overnight at 4°C. Four baits were collected from different places of survey which include

- Hairs of pig
- Hairs of goat.
- Human nail.
- Feather of hen.

Defatting of baits was done by soaking them for 24 hrs in either diethyl ether and later rinsing them 4-5 times with distilled water followed by air drying.

Isolation & identification of keratinophilic fungi:

Keratinophilic fungi were isolated by the hair baiting technique of Vanbreuseghem (Vanbreuseghem,



Scheme 1. Hair baiting technique for isolation of keratinophilic fungi.

1952) using autoclaved human nail, pig hair, chicken feather and goat hair as keratin bait (Scheme 1). For this, sterile Petri dishes half filled with the soil samples and moistened with sterile distilled water

were baited by burying sterile keratinous bait in the soil. These dishes were incubated at room temperature and examined daily from the third day for fungal growth over a period of 4 weeks. After observing the mycelial growth on the baits, isolates were cultured on Sabouraud's Dextrose Agar (SDA) medium supplemented with streptomycin (30 mcg/l). These fungi were identified on the basis of the monographs of Sigler and Carmichael, 1976; Oorchschot, 1980; Currah, 1985; Von Arx, 1986; Sigler et al., 1986; Cano and Guarro, 1990, 1994; Vidal et al., 2002 and Sigler et al., 2002 by using macro and micro-morphological character of these cultures.

Results and Discussion

Present study shows an overall prevalence of keratinophilic fungi in burning ghat soils at Ranchi, Jharkhand. Data on the distribution of these fungi in garbage waste soils is presented in Table 1. A total of 107 colonies of different keratinophilic fungi were isolated from 48 soil samples. The isolated keratinophilic fungi were classified into 9 species belonging to 6 genera (Fig. 1). The isolated keratinophilic fungi were in the following order of dominance: *Rhizopus stolonifer* and *Aspergillus niger* (14.02%), *Mucor pucillus* (13.08%), *Chrysosporium* sp. (12.15%), *F. Oxysporum* (11.2%), *A. Terreus* and *Penicillium chrysogenum* (9.34%) and *A. flavus* and *Penicillium* sp. (8.41%).

Table 1. Distribution of keratinophilic fungi in burning ghat soils at Ranchi

Sl. No.	Isolate identity	Sampling Site (Blocks)												Total colonies	% occurrence
		1	2	3	4	5	6	7	8	9	10	11	12		
1.	<i>Aspergillus niger</i>	1	2	1	3	-	2	1	-	2	1	1	2	15	14.02
2.	<i>A. terreus</i>	-	1	-	2	2	1	-	-	-	2	1	1	10	9.34
3.	<i>A. flavus</i>	2	-	1	1	1	-	1	1	-	1	-	1	9	8.41
4.	<i>Penicillium chrysogenum</i>	1	2	-	1	3	1	-	-	-	1	1	-	10	9.34
5.	<i>Penicillium sp.</i>	1	1	1	1	1	-	-	1	1	-	1	1	9	8.41
6.	<i>Fusarium oxysporum</i>	2	1	-	-	1	2	2	-	-	1	1	2	12	11.21
7.	<i>Chrysosporium sp.</i>	2	1	2	1	-	-	2	1	-	2	1	1	13	12.15
8.	<i>Mucor pusillus</i>	3	1	-	1	2	1	-	1	1	2	1	1	14	13.08
9.	<i>Rhizopus stolonifer</i>	1	-	2	1	1	3	1	2	-	1	2	1	15	14.02
Total colonies		13	9	7	11	11	10	7	6	4	11	9	10	107	

Frequency of occurrence of the keratinophilic fungi (Table 2.) is in the order *A.niger*, *R. Stolonifer* and *Mucorpusillus* (83.33%), *Chrysosporium sp.* And *Penicillium sp.* (75.00%), *A. Flavus* and *F. Oxysporum* (66.67%) & *A. Terreus* and *P. Chrysogenum* (58.33%).

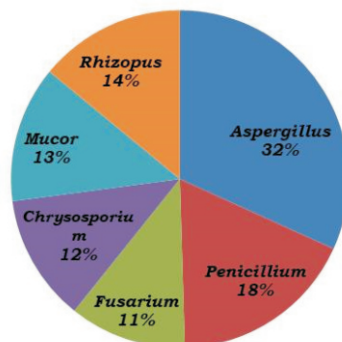
Table 2. Frequency occurrence of keratinophilic fungi in burning ghat soils at Ranchi

Sl. No.	Name of the isolate	Number	% value
1.	<i>Aspergillus niger</i>	10	83.33
2.	<i>A. terreus</i>	7	58.33
3.	<i>A. flavus</i>	8	66.67
4.	<i>Penicillium chrysogenum</i>	7	58.33
5.	<i>Penicillium</i> sp.	9	75.00
6.	<i>Fusarium oxysporum</i>	8	66.67
7.	<i>Chrysosporium</i> sp.	9	75.00
8.	<i>Mucor pusillus</i>	10	83.33
9.	<i>Rhizopus stolonifer</i>	10	83.33

Fungi isolated on different keratinaceous baits are presented in Table 3.

Table 3. Keratinophilic fungi isolated on different baits

Sl. No.	Name of the fungus	Baits			
		Human nail	Pig Hair	Chicken feather	Goat hair
1.	<i>Aspergillus niger</i>	+	+	+	+
2.	<i>A. terreus</i>	-	+	-	-
3.	<i>A. flavus</i>	+	+	+	-
4.	<i>Penicillium chrysogenum</i>	+	-	+	+
5.	<i>Penicillium</i> sp.	-	-	-	+
6.	<i>Fusarium oxysporum</i>	+	+	-	-
7.	<i>Chrysosporium</i> sp.	+	-	-	+
8.	<i>Mucor pusillus</i>	-	+	+	+
9.	<i>Rhizopus stolonifer</i>	-	+	-	-

**Fig. 1. Keratinophilic genus isolated from burning ghat soils**

Keratinophilic fungi play an important role in the natural degradation of keratinized residues in the soil and the keratinolytic activity of fungi was important ecologically and had attracted the attention of researchers throughout the world (Fillipello, 2000; Sharma and Rajak, 2003; Zarrin and Haghgoo, 2011). The presence of keratinophilic fungi in different soil has been reported worldwide (Shadzi *et al.*, 2002; Deshmukh, 2004; Saxena *et al.*, 2004; Zarei and Zarrin, 2008; Shrivastava *et al.*, 2008). These fungi are associated with human and animal mycoses (Fillipello *et al.*, 1996 and Spiewak and Szostak, 2000). But there are no reports on the presence of keratinophilic fungi from the burning ghat soils in India and the world. Although the fungi isolated are commonly of non-dermatophytic in nature, but some of the isolates are found to be pathogenic to humans. During the present study *Aspergillus niger*, *Rhizopus stolonifer* and *Mucor pucillus* were most prevalent isolate both in terms of its percent occurrence and frequency of occurrence. Several reports indicated that *Penicillium* was the most prevalent saprophyte isolated during their study on keratinophilic fungi (Shokohi *et al.*, 2005 and Zarrin and Haghgoo, 2011).

Study showed that the genus *A. niger*, one of the dominant fungus in the burning ghat soils of Ranchi, is pathogenic to humans and cause aspergillosis and may also cause pulmonary disease in immunocompromised patients and the production of oxalate crystals in clinical specimens (Nakagawa *et al.*, 1999). *A. flavus*, also isolated during the present study, is reported to have keratinase activity. This possibly describes the recovery of fungus from the sterile hair bait. *A. flavus* had been recognised as a strong producer of extracellular keratinase in medium including porcine nail as the source of nitrogen and carbon (Anbu *et al.*, 2006; Zarrin and Haghgoo, 2011). One species of *Fusarium* has also been isolated, which is described to be active in extracellular keratinases after grown on agar including soluble keratin (Anbu *et al.*, 2006). Presence of *Rhizopus*, *Mucor*, *Trichoderma* and *Curvularia* species in various soil samples have also been reported by various workers (Anbu *et al.*, 2004; Zarrin *et al.*, 2011; Avasthi *et al.*, 2012).

The occurrence of *Chrysosporium* sp. in garbage waste soils is an important finding of present study as pathogenic potential of this fungus and was confirmed in several studies in different countries. For instance, *C. zonatum* was shown causing systemic infection in a person with a chronic granulomatous disease (Garg, 1996; Roilides *et al.*, 1999; Ulfig, 2006). Various species of *Chrysosporium* have been reported from Indian soils (Kushwaha and Agrawal, 1976; Nigam and Kushwaha, 1989; Deshmukh, 2004; Deshmukh *et al.*, 2010).

The present research reports for the first time the existence of keratinophilic fungi in the burning ghat soils of Ranchi, India. Therefore hygiene protocol should be taken to prevent the spread of pathogenic fungi in these environments as there is a risk of fungal infections of human.

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