

Isolation and identification of keratinophilic fungi from garbage waste soils of Jharkhand region of India

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ABSTRACT

*Forty eight soil samples were collected from 12 garbage waste 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 64 colonies of different keratinophilic fungi were isolated from 48 soil samples. The isolated keratinophilic fungi were classified into 10 species belonging to 7 genera. The isolated keratinophilic fungi were in the following order of dominance: *Penicillium chrysogenum* (15.62%), *Aspergillus niger* and *Rhizopus stolonifer* (14.06%), *Trichoderma harzianum* (12.50%), *Fusarium oxysporum* (10.94%), *Aspergillus sp.1* (7.81%), *Aspergillus flavus* and *Curvularia lunata* (5.97%), *Penicillium sp.* (5.81%) & *Chrysosporium sp.* (4.69%).*

Key words: Piggery, Hair baiting technique, Keratinophilic fungi, Dominance.

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. 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. Studies on Keratinophilic fungi started in 1952 with the invention of the technique of the isolation soil fungi and soil proved to be the natural reservoir of these fungi. The potentially pathogenic keratinophilic fungi and allied geophilic dermatophytic species are widespread worldwide. Reports on the presence of these fungi in different soil habitats from different countries e.g. Egypt[1], Australia[2], Palestine[3], Spain [4], India[5-8], Korea[9], Iran[10], Kuwait[11], and Malaysia[12] have shown the fact of its worldwide distribution. Some of these fungi are well-known dermatophytes and are known to cause superficial cutaneous infections (dermatophytoses) of keratinized tissues (skin, hair and nails) of humans and animals.

A few investigators have reported the occurrence of dermatophytes and other keratinophilic fungi from various habitats in India [8, 13-18]. 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 garbage waste soils of Jharkhand state of India.

MATERIALS AND METHODS

Collection of soil samples and baits:

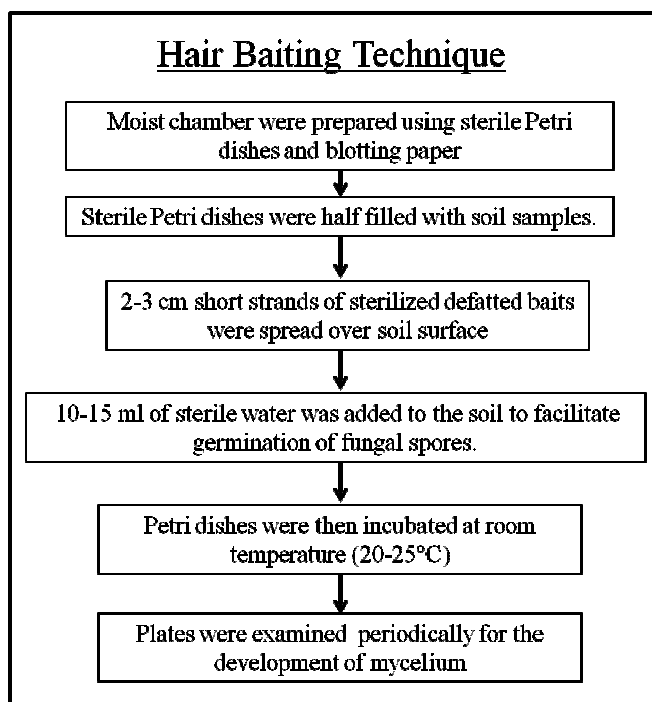
Forty eight soil samples were collected randomly from 12 garbage dumping sites (4 samples per site) from the Ranchi district of Jharkhand. Before collection of soil samples, superficial debris and other vegetative materials were removed from the soil surface. Loosened soil (approximately 500g) were taken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

- a. Hairs of pig
- b. Hairs of goat.
- c. Human nail.
- d. 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 [19] 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 mg/l).



Scheme 1. Hair baiting technique for isolation of keratinophilic fungi

Isolated fungi were stained with Lactophenol cotton blue and observed it under the phase contrast microscope ((type 020-519.503 LB 30T, Leica, Germany) and were identified on the basis of the monographs of Sigler and Carmichael[20], Oorschot [21], Currah[22], Von Arx [23], Sigler *et al.*, [24], Cano and Guarro [25], Cano *et al.*, [26], Vidal *et al.*, [27] and Sigler *et al.*, [28] by using macro and micro-morphological character of these cultures.

RESULTS AND DISCUSSION

Present study shows an overall prevalence of keratinophilic fungi in garbage waste soils at Ranchi, Jharkhand. Data on the distribution of these fungi in garbage waste soils is presented in Table 1.

Table 1. Distribution of keratinophilic fungi in garbage waste soils at Ranchi

Sl. No.	Name of the fungus	Sampling Site												Total colonies	% occurrence
		1	2	3	4	5	6	7	8	9	10	11	12		
1.	<i>Aspergillus niger</i>	1	1	1	-	1	2	-	1	-	1	1	-	9	14.06
2.	<i>A. flavus</i>	1	-	-	-	-	1	1	-	-	-	-	1	4	5.97
3.	<i>Aspergillus</i> sp.1	-	1	1	-	-	-	-	1	1	-	-	1	5	7.81
4.	<i>Penicillium chrysogenum</i>	2	1	-	1	2	1	-	-	1	1	-	1	10	15.62
5.	<i>Penicillium</i> sp.	1	-	-	1	-	-	-	-	1	2	-	-	5	5.81
6.	<i>Fusarium oxysporum</i>	1	2	-	1	-	-	-	1	1	-	1	-	7	10.94
7.	<i>Trichoderma harzianum</i>	1	-	-	-	1	2	1	-	-	1	1	1	8	12.50
8.	<i>Curvularia lunata</i>	1	-	1	-	1	-	-	-	-	-	1	-	4	5.97
9.	<i>Chrysosporium</i> sp.	-	-	-	-	1	-	-	1	-	-	-	1	3	4.69
10.	<i>Rhizopus stolonifer</i>	1	2	-	1	1	-	-	1	1	1	-	1	9	14.06
Total colonies		9	7	3	4	7	6	2	5	4	4	6	6	64	

A total of 64 colonies of different keratinophilic fungi were isolated from 48 soil samples. The isolated keratinophilic fungi were classified into 10 species belonging to 7 genera. The isolated keratinophilic fungi were in the following order of dominance: *Penicillium chrysogenum* (15.62%), *Aspergillus niger* and *Rhizopus stolonifer* (14.06%), *Trichoderma harzianum* (12.50%), *Fusarium oxysporum* (10.94%), *Aspergillus* sp.1 (7.81%), *Aspergillus flavus* and *Curvularia lunata* (5.97%), *Penicillium* sp. (5.81%) & *Chrysosporium* sp. (4.69%). Frequency of occurrence of the keratinophilic fungi (Table 2.) is in the order *Aspergillus niger*, *Penicillium chrysogenum* and *Rhizopus stolonifer* (66.67%), *Trichoderma harzianum* (58.33%), *Fusarium oxysporum* (50.00%), %, *Aspergillus* sp.1 (41.67%), *Aspergillus flavus*, *Curvularia lunata* and *Penicillium* sp. (33.33%) and minimum in *Chrysosporium* sp. (25.00%). Fungi isolated on different keratinaceous baits are presented in Table 3.

Table 2. Frequency occurrence of keratinophilic fungi in garbage waste soils at Ranchi

Sl. No.	Name of the Fungus	Number	% value
1.	<i>Aspergillus niger</i>	8	66.67
2.	<i>A. flavus</i>	4	33.33
3.	<i>Aspergillus</i> sp.1	5	41.67
4.	<i>Penicillium chrysogenum</i>	8	66.67
5.	<i>Penicillium</i> sp.	4	33.33
6.	<i>Fusarium oxysporum</i>	6	50.00
7.	<i>Trichoderma harzianum</i>	7	58.33
8.	<i>Curvularia lunata</i>	4	33.33
9.	<i>Chrysosporium</i> sp.	3	25.00
10.	<i>Rhizopus stolonifer</i>	8	66.67

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. flavus</i>	-	+	-	-
3.	<i>Aspergillus</i> sp.1	+	+	+	-
4.	<i>Penicillium chrysogenum</i>	+	-	+	+
5.	<i>Penicillium</i> sp.	-	-	-	+
6.	<i>Fusarium oxysporum</i>	+	+	-	-
7.	<i>Trichoderma harzianum</i>	+	+	-	+
8.	<i>Curvularia lunata</i>	-	+	+	-
9.	<i>Chrysosporium</i> sp.	+	-	-	-
10.	<i>Rhizopus stolonifer</i>	+	+	-	+

The presence of keratinophilic fungi in different soil has been reported worldwide [17, 29-32]. Keratinolytic activity of fungi is important ecologically and has attracted the attention of researchers throughout the world [33-35]. These fungi are associated with human and animal mycoses [36-37]. 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 *Penicillium chrysogenum* was 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 [35, 38]. Study showed that the genus *A. niger*, one of the dominant fungus in the garbage waste 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 [39]. *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 [35, 40]. One species of *Fusarium* has also been isolated, which is

described to be active in extracellular keratinases after grown on agar including soluble keratin [40]. Several reports have indicated that *Aspergillus* species are among the most prevalent keratinophilic fungi in the soils [41-45]. Presence of *Rhizopus*, *Mucor*, *Trichoderma* and *Curvularia* species in various soil samples have also been reported by various workers [6, 35, 46]. 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 showed causing systemic infection in a person with a chronic granulomatous disease [47-49]. Various species of *Chrysosporium* have been reported from Indian soils [8, 13, 17, 50-51].

The present research reports for the first time the existence of keratinophilic fungi in the garbage waste 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. These findings should be taken into consideration and necessary treatment methods should be taken up periodically.

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