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PREVALENCE OF KERATINOPHILIC FUNGI IN PIGGERY SOILS OF JHARKHAND, INDIA

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ABSTRACT

Jharkhand state of India has been a home to a variety of tribal communities comprising total of 32 different tribal groups constituting around 28% of the total population of state. Tribal population in this area use to run piggeries and also pork is an important part of their fooding habit. Forty eight soil samples were collected from 12 piggeries 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. Fifteen isolates belonging to 9 genera were recovered and identified by recognition of their macro- and micro-morphological features. The isolated species were reported in the following order of dominance: *Aspergillus niger* (12.06%), *Fusariun oxysporum* (10.34%), *Penicillium chrysogenum* and *Mucor pusillus* (8.62%), *Trichoderma harzianum*, *Penicillium* sp. and *Rhizopus stolonifer* (8.04%), *Curvularia lunata and Aspergillus terreus* (5.17%), *Aspergillus flavus*, *Fusarium solani*, *Trichoderma reesei* and *Alternaria alternata* (4.59%), *Chrysosporium* sp. (4.02%) and *Trichoderma viride* (3.44%).

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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. 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 inhibit 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. Most keratinophilic fungi belong to families Arthodermataceae and Onygenaceae of the order Onygenales in Ascomycetes (Currah, 1985). Keratinophilic fungi represent an important component of soil microflora where they decompose the highly resistant keratin. Frequency of occurrence of keratinophilic fungi in the soil is influenced by a number of biotic and abiotic factors (Otsenasek, 1978). Reports on the presence of these fungi in different soil habitats from different countries e.g. Egypt (All *et al.*, 1987), Australia (Rose, 1980), Palestine (Ali-Shtayeh, 1989), Spain (Clavo *et al.*, 1984), India (Pandey *et al.*, 1989; Anbu *et al.*, 2004; Ganaie, 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 that these groups of fungi have its distribution worldwide. The presence of keratinophilic in environment is variable and is influenced by human and animal interference (Mearcantini *et al.*, 1983). Many investigations have been carried out in recent years on the distribution of keratinophilic fungi (Ajello *et al.*, 1965; Al-Doory, 1967; Guarro *et al.*, 1981; Filipello, 1986, Agut *et al.*, 1995; Simpanya *et al.*, 1996; Caretta *et al.*, 1975; Papini *et al.*, 1998; Deshmukh, 1999; Deshmukh *et al.*, 2010; Lee *et al.*, 2011). The keratinophilic fungi inhibiting soil have special affinity to keratinous substrates and are potentially pathogenic to human and animals (Mitra, *et al.*, 1998). These fungi have attracted the attention of mycologists and dermatologists all over the world due to their association with human and animal mycoses (Rippon *et al.*, 1969; Mirocha *et al.*, 1972). In Jharkhand, tribal population use to consume pork on a large scale and thus there are lot of piggeries in the state. Hence it is significant to analyse the microflora of piggeries in Jharkhand in order to evaluate the presence and extent of keratinophilic fungi in these environments. A few investigators have reported the occurrence of dematophytes and other keratinophilic fungi from various habitats in India (Singh *et al.*, 1994; Ramesh and Tilda, 1999; Deshmukh, 2002; Deshmukh and Verepar, 2006). 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 soils collected from pig farms in the state.

MATERIALS AND METHODS

Forty eight soil samples were collected randomly from 12 pig farms (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. Keratinophilic fungi were isolated by the hair baiting technique of Vanbreuseghem (Vanbreuseghem, 1952) using autoclaved human nail, pig hair, chicken feather and goat hair as keratin bait. 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). These fungi were identified on the basis of the monographs of Sigler and Carmichael, 1976; Oorschot, 1980; Currah, 1985; Von Arx, 1986; Sigler *et al.*, 1986; Cano and Guarro, 1990; Cano *et al.*, 1994; Vidal *et al.*, 2002 and Sigler *et al.*, 2002 by using macro and micro-morphological character of these cultures.

RESULTS AND DISCUSSION

Data on the distribution of keratinophilic fungi in piggery soils at Ranchi, Jharkhand is presented in Table 1. A total of 174 colonies of different keratinophilic fungi were isolated from 48 soil samples. The isolated keratinophilic fungi were classified into 15 species belonging to 9 genera. The isolated keratinophilic fungi were in the following order of dominance: *Aspergillus niger* (12.06%), *Fusariun oxysporum* (10.34%), *Penicillium chrysogenum* and *Mucor pusillus* (8.62%), *Trichoderma harzianum*, *Penicillium* sp. and *Rhizopus stolonifer* (8.04%), *Curvularia lunata and A. terreus* (5.17%), *A. flavus*, *Fusarium solani*, *T. reesei* and *Alternaria alternata* (4.59%), *Chrysosporium* sp. (4.02%) and *T. viride* (3.44%). Frequency of occurrence of the keratinophilic fungi (Table 2; Fig.1.) is in the order *Mucor pusillus* and *Fusariun oxysporum* (833.33%), *A. niger* and *Penicillium chrysogenum* (75%), *T. harzianum* and *Penicillium* sp. (66.77%), *A. flavus* and *Rhizopus stolonifer* (58.33%), *Curvularia lunata*, *T. reesei*, *Alternaria alternate*, *Fusarium solani*, *Chrysosporium* sp. *and A. terreus* (50%) and *T. viride* (41.66%). Fungi isolated on different kearatinaceous baits are presented in Table 3.

Table 2: Frequency occurrence of keratinophilic fungi

S. No.	Name of the Fungus	Number	% value
1.	*Aspergillus niger*	9	75.00
2.	*A. terreus*	6	50.00
3.	*A. flavus*	7	58.33
4.	*Penicillium chrysogenum*	9	75.00
5.	*Penicillium* sp.	8	66.67
6.	*Alternaria alternata*	6	50.00
7.	*Fusariun oxysporum*	10	83.33
8.	*F. solani*	6	50.00
9.	*Trichoderma harzianum*	8	66.67
10.	*T. reesei*	6	50.00
11.	*T. viride*	5	41.66
12.	*Curvularia lunata*	6	50.00
13.	*Chrysosporium* sp.	6	50.00
14.	*Mucor pusillus*	10	83.33
15.	*Rhizopus stolonifer*	7	58.33

Table 3: Keratinophilic fungi isolated on different baits

Name of the fungus	Baits			
	Human nail	Pig hair	Chicken feather	Goat hair
Aspergillus niger	+	+	+	+
A. terreus	-	+	-	-
A. flavus	+	+	+	-
Penicillium chrysogenum	+	-	+	+
Penicillium sp.	-	-	-	+
Alternaria alternata	+	+	-	-
Fusariun oxysporum	+	+	-	+
F. solani	-	+	+	-
Trichoderma harzianum	+	-	+	+
T. reesei	-	+	-	+
T. viride	+	+	+	-
Curvularia lunata	+	-	+	-
Chrysosporium sp.	+	-	-	+
Mucor pusillus	-	+	+	+
Rhizopus stolonifer	-	+	-	+

Keratinophilic fungi play an important role in the natural degradation of keratinized residues in the soil and the keratinolytic activity of fungi is important ecologically and has attracted the attention of researchers throughout the world (Fillpello, 2000; Sharma and Rajak, 2003; Zarrin and Haghgoo, 2011). These fungi may cause human and animal infections

Table 1: Distribution of keratinophilic fungi in piggery soils at Ranchi

S. No.	Name of the fungus	Sampling site											Total	%
		1	2	3	4	5	6	7	8	9	10	11	12	colonies	occurrence
1.	*Aspergillus niger*	2	1	4	-	3	-	1	1	2	-	4	3	21	12.06
2.	*A. terreus*	-	2	1	-	-	1	2	-	1	-	2	-	9	5.17
3.	*A. flavus*	1	-	-	-	2	-	1	-	1	1	1	1	8	4.59
4.	*Penicillium chrysogenum*	2	2	3	1	-	1	2	1	-	-	2	1	15	8.62
5.	*Penicillium* sp.	1	2	3	3	1	-	-	-	1	-	1	2	14	8.04
6.	*Alternaria alternata*	-	-	1	1	2	1	-	1	-	-	2	-	8	4.59
7.	*Fusariun oxysporum*	-	3	2	1	4	2	1	1	1	2	-	1	18	10.34
8.	*F. solani*	1	-	-	-	1	2	-	2	1	1	-	-	8	4.59
9.	*Trichoderma harzianum*	3	2	1	-	2	1	-	2	-	2	-	1	14	8.04
10.	*T. reesei*	-	-	1	-	-	-	1	-	1	2	2	1	8	4.59
11.	*T. viride*	-	1	2	1	-	-	-	-	1	-	1	-	6	3.44
12.	*Curvularia lunata*	1	-	-	-	1	2	1	3	1	-	-	-	9	5.17
13.	*Chrysosporium* sp.	2	-	1	1	1	-	1	1	-	-	-	-	7	4.02
14.	*Mucor pusillus*	2	3	1	1	-	2	1	1	-	1	2	1	15	8.62
15.	*Rhizopus stolonifer*	3	-	-	2	1	-	2	2	1	3	-	-	14	8.04
Total colonies		18	16	20	11	18	12	13	15	11	12	17	11	174	

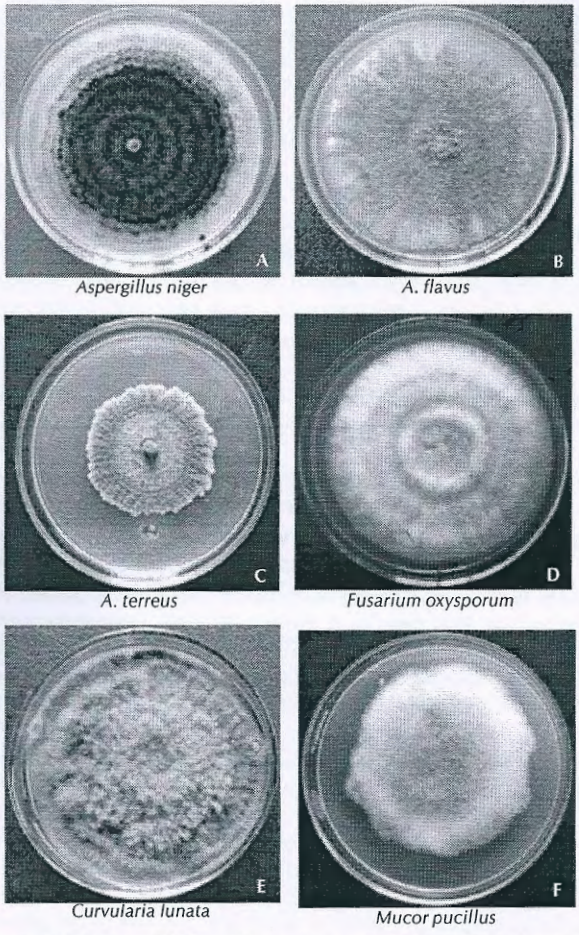

Figure 1: Cultures of different kearatinaceous isolates from piggeries of Ranchi, Jharkhand

(Connole, 1990; Fillipello *et al.*, 1996; Spiewak and Szostak, 2000). The presence of these fungi in different soil has been reported from different countries (Shadzi *et al.*, 2002; Deshmukh, 2004; Saxena *et al.*, 2004; Zarei and Zarrin, 2008; Shrivastava *et al.*, 2008). The present study also showed the presence of keratinophilic fungi in the soil (piggery) of Ranchi, Jharkhand. Although the fungi isolated are commonly non-dermatophytic, but some of the isolates are found to be pathogenic to humans. Study showed that the genus *A. niger*, one of the dominant fungus in the piggery 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; Steinbach and Stevens, 2003). Two species of *Fusarium* were also isolated from the piggery soils of Ranchi. Some strains of *Fusarium* were described to be active in extracellular keratinoases after grown on agar including soluble keratin (Anbu *et al.*, 2006).

During the present study *Penicillium chrysogenum* and *Penicillium* sp. were also prevalent. Several reports indicated that *Penicillium* was the most prevalent saprophyte isolated during their study on keratinophilic fungi (Shokohi *et al.*, 2005; Zarrin and Haghgoo, 2011). The occurrence of *Chrysosporium* sp. in piggery 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 (Garg, 1996; Roilides *et al.*, 1999; Ulfig, 2006). Various species of *Chrysosporium* have been reported from Indian soils (Kushwaha and Agrawal, 1976; Nigam *et al.*, 1989; Singh *et al.*, 1994; Deshmukh, 2004; Deshmukh *et al.*, 2010). *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 *et al.*, 2011). Hubalek *et al.* 1973; Hubalek, 1974 have reported the occurrence of *Aspergillus* species from bird's feathers. Presence of *Rhizopus*, *Mucor*, *Trichoderma*, *Curvularia* and *Alternaria* species in various soil samples have also been reported by various workers (Anbu *et al.*, 2004; Zarrin *et al.*, 2011; Avasn *et al.*, 2012).

The present research shows the existence of keratinophilic fungi in the piggery 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 in and around the piggeries as the tribal population in this area use to run piggeries and also pork is an important part of their fooding habit.

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