

Short Communication

Isolation of Keratinophilic Fungi from Soil in Khairpur City, Sindh, Pakistan

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One hundred and twenty five soil samples were collected from five areas in the vicinity of Khairpur city, Sindh, Pakistan and screened for the presence of keratinophilic fungi by using hair bait technique. A total 253 isolates belonged to eight genera and eleven species of keratinophilic fungi were recovered. Maximum number (153/253, 60.5%) of keratinophilic fungi was recovered from farm land and poultry soils. *Aspergillus niger* was most prevalent species and represented 20.2% of the total number isolated. *A. flavus* and *A. fumigatus* were almost equal in their prevalence and represented 11.9 and 11.1%, respectively. *Botrytis cinaria* comes next constituting 9.9%; followed by *Mucor* spp. (9.1%) and *Chochliobolus lunatus* (8.3%). Other species isolated less frequently included *Chrysosporum asperatum* (5.9%), *Fusarium* spp. (5.1%), *Penicillium* spp. (3.1%) and *A. wentii* (2.4%). The distribution pattern of the different keratinophilic fungi and their significance are discussed.

Keywords: Keratinophilic fungi, Prevalence, Keratinase, Hair bait technique

Keratinophilic fungi like to grow and even reproduce on keratin materials such as skin, hair, nail, fur, feather, horn, hoof beak of the birds etc. They utilize keratin as carbon source¹. Keratin is highly insoluble protein having fibrous helical structure and numerous disulfide linkages which make it resistant to many proteases but is easily digested by keratinase enzymes².

The kerateneous materials in or on soil are attacked by these keratinophilic microbes, therefore biodegradation takes place. Keratinases also provide the virulence to certain fungi such as dermatophytes to cause dermatophytoses or ringworm in human and animals³⁻⁴. These enzymes, on the other hand, have immense effect on prion which causes bovine spongy encephalitis (BSE) commonly known as 'mad cow' disease because these enzymes successfully digested and removed the prion from diseased tissue of brain⁵. There are also other applications of keratinases in various industries such as leather, poultry, cosmetics, diagnostics, and pharmaceuticals⁶⁻⁹.

Keeping in view such enormous significance of keratinophilic fungi for keratinase enzymes production, the present study was under taken to isolate these microorganisms from fertile soils, animal herds, animal slaughter houses, poultries and barbers' shops. A total 125 soil samples were collected from five different sources including fertile lands, animal herds, animal slaughter houses, poultries and barbers' shops in Khairpur city, Sindh, Pakistan. From each sources 25 soil samples were collected and screened for keratinophilic fungi by employing hair bait technique using human hair as keratin bait¹⁰⁻¹⁷. Invaded hairs were inoculated on Sabourauds dextrose agar (SDA) supplemented

with chloramphenicol 0.05 mg/l for obtaining the cultural growth. Cultures were purified and maintained on potato dextrose agar (PDA) at 5°C. The identification of isolates was made as per Dexter³ and Domesck *et al.*¹.

The results of the isolation of keratinophilic fungi are presented in Table 1. The data revealed that of 125 samples, maximum number (85/253; 33.6%) of keratinophilic fungi was isolated from soils of fertile lands; followed by the soil samples from poultries (68/253; 26.9%). Isolation rates of keratinophilic fungi from soils of animal herd (44/253; 17.4%) and barbers' shops (43/253; 17.0%) were almost similar. The least number of keratinophilic fungi was isolated from the slaughter house soils (13/253; 5.1%).

A total of 253 keratinophilic fungi including eight genera and eleven species were isolated, *viz.*, *Aspergillus niger* (20.2%), *Alternaria alternata* (13.0%), *Aspergillus flavus* (11.9%), *Aspergillus fumigatus* (11.1%), *Botrytis cinaria* (18.2%), *Chochliobolus lunatus* (16.8%), *Mucor* spp. (16.0%), *Chrysosporum asperatum* (9.9%), *Fusarium* spp. (5.1%), *Penicillium* spp. (3.1%) and *Aspergillus wentii* (2.4%) (Table 1). *Aspergillus* accounted for 45.6% distribution, with *A. niger* being the dominant species. It is interesting that some fungi isolated in this study such as *Aspergillus*, *Alternaria*, *Chochliobolus*, *Botrytis*, *Fusarium* and *Mucor* were previously thought as non-keratinophilic, but subsequent studies showed that these fungi possess keratinolytic activities when grown on keratin-rich substrates and they also occur in the environment in keratin-rich soils^{13,15-17}. Keratinolytic enzymes have been shown to be useful for biotechnological purposes such as hydrolysis of poultry feathers¹⁹ and dehairing of bovine pelts²⁰.

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Table 1. Isolation rate keratinophilic fungi from various soils collected in Khairpur city

Keratinophilic fungus	Isolation rate, No. (%)					
	Fertile land (n = 25)	Animal herd (n = 25)	Slaughter house (n = 25)	Poultry (n = 25)	Barbers' shop (n = 25)	Total (n = 125)
<i>Alternaria alternata</i>	8 (32)	2 (8)	1 (4)	13 (52)	9 (36)	33 (13.0)
<i>Aspergillus niger</i>	15 (60)	7 (28)	2 (8)	18 (72)	9 (36)	51 (20.2)
<i>Aspergillus flavus</i>	10 (40)	4 (16)	1 (4)	5 (20)	10 (40)	30 (11.9)
<i>Aspergillus fumigatus</i>	10 (40)	3 (12)	0 (0)	11 (44)	4 (16)	28 (11.1)
<i>Aspergillus wentii</i>	3 (12)	1 (4)	0 (0)	2 (8)	0 (0)	6 (2.4)
<i>Botrytis cinaria</i>	10 (40)	7 (28)	0 (0)	5 (20)	3 (12)	25 (9.9)
<i>Chochliobolus lunatus</i>	4 (16)	6 (24)	1 (4)	5 (20)	5 (20)	21 (8.3)
<i>Chrysosporum asperatum</i>	2 (8)	9 (36)	3 (12)	0 (0)	1 (4)	15 (5.9)
<i>Fusarium</i> species	9 (36)	1 (4)	1 (4)	2 (8)	0 (0)	13 (5.1)
<i>Mucor</i> species	11 (44)	4 (16)	4 (16)	3 (12)	1 (4)	23 (9.1)
<i>Penicillium</i> species	3 (12)	0 (0)	0 (0)	4 (16)	1 (4)	8 (3.1)
Total	85 (33.6)	44 (17.4)	13 (5.1)	68 (26.9)	43 (17.0)	253 (100.0)

It appears from this study that *Aspergillus niger* is the most prevalent keratinophilic fungus and also dominant species that isolated from 51 soil samples of five different regions like fertile lands, animal herds, slaughter houses, poultries and barbers' shops. Isolation rate of keratinophilic fungi including *A. niger* was higher in soil samples collected from the farm lands and poultries. Keratinases have enormous potential applications in processing waste in the poultry and leather industries. In this study, some keratinophilic fungi were isolated, which could be used for the production of adequate amounts for application in industrial processes.

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