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Existence of Keratinophilic Fungi in the Soils of Hilly and Plain Areas

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Abstract: Total of 120 samples, 60 from each site were collected from the soil of Shimla (H.P.) and Agra (U.P.). Collected soil samples were baited for the isolation of fungi capable of colonization and attacking keratinous substrates. Out of 60 samples of both sites, 47 were recorded positive for hilly and 34 for plain. The percentage prevalence of fungal species in hilly and plain areas was calculated 78.3 % and 56.6 % respectively. Only four species from non-keratinophilic fungal species were isolated i.e. *Aspergillus niger, Mucor* sps, *Rhizopus* sps and *Fusarium* sps in hilly areas whereas from plain areas only *Aspergillus niger* and *Mucor* sps were isolated. One species each of *Cladosporium, Emmonsia, Geomyces* and *Zymonema* and five species each of genera *Chrysosporium, Trichophyton and Microsporum* were isolated from both the areas.

Keywords: Baited, Keratinophilic Fungi, Isolation, Keratinous, Prevalence

Introduction

Innumerable microorganism (algae, viruses, mycoplasma) mostly fungi and bacteria have been identified and reported to utilize keratin as substrates by many authors (Abdel Hafez and El- Sharoumy 1990; Malviya *et al* 1992; Simpanya and Baxter 1996; Singh 1997). Keratinophilic are the important group of fungi that usually are small, well defined and colonize various keratinous substrates and degrades them to components of low molecular weight. Keratin is a proteinaceous substance of animal origin, which consists of polypeptide chain jointed by hydrogen bonds, salt cross bridges and disulphide bridges. It occurs in nature in various forms as in animals and human being in form of appendages like hair, wool, feather, nails, hooves, horn and also on the outer keratin layer of skin which differ from proteins in its higher cysteine content. The majority of fungi (*Chrysosporium, Microsporum, Epidermophyton, Trichophyton, Geomyces, Myceliophthora* etc), which are able to decompose keratin, form a group that have a no. of common morphological and physiological characters and are members of the primitive Ascomycetes fungi. Soil provides an antagonistic medium for keratinophilic fungi and related fungi including *Fusarium,*

Aspergillus, Penicillium and Trichoderma. A no. of biotic and abiotic factors influence the frequency of occurrence of keartinophilic fungi in the soil (Otsenasek, 1978). Various reports have been found available on the presence of fungi in different soil habitats from different countries i.e. Australia (Rose *et. al.* 1980), Palesteine (Ali- Shtayeh 1989), India (Ganaei 2010, Anbu *et. al.* 2004, Pandey *et. al.* 1989, Deshmukh *et. al.* 2010), Iran (Mahmoudabadi *et. al.* 2008), Spain (Clavo *et. al.* 1984), Kuwait (Al- Musallam 1989 and Mahmoudabadi *et. al.* 2008), Egypt (All *et. al.* 1987), Malaysia (Soon 1991) and Korea (Lee *et. al.* 2011). Dermatophytes and other keratinophilic fungi have been reported to occur in several habitats of India (Shukla and Chouhan *et. al.* 2011, Kushwaha & Agrawal 1976, Deshmukh 2002, Ramesh and Tilda, 1999, Singh *et. al.* 1999). Feathers represent 5-7% of body weight of the domestic fowl. Poultry feather locks up a great potentiality of being useful protein and amino acids that could be beneficial, harnessed as animal foodstuff. Since there are no reports available on Keratinophilic fungi in Shimla and Agra, India. The present study was carried out in which keratinophilic fungi were isolated from the soil of different sites i.e. from hilly and plain areas.

Materials and methodology

Collection of soil samples

120 Soil samples were collected from gardens, cattles yards, hospitals, poultry farms, open fields of two regions (Shimla and Agra) with the help of spatula in sterile polythene bags and stored at 4˚C. Soil samples from plain areas were collected from surface of the soil itself only upto the depth of 3-4 inches while from the hilly areas the soil were collected from the superficial layer at an altitude of 2500 metres.

Isolation of keratin decomposing fungi

Using feather as baits, keratin decomposing fungi were isolated from chicken feathers by To-Ka-Va hair bating technique of Bendeck (1962). Chicken feathers were used as substrates which were collected from the poultry farms of Agra, India and were sterilized as per the procedure of Evans and Hose (1975). The feather were cut into pieces, washed with sterilized water and autoclaved for 10 min at 10- lbs/m^3 pressure and used as substrate.

All the soil samples were incubated with above prepared baits at 28±2˚C in an incubator. Visible mycelium appeared on baits which was microscopically observed by mounting in cotton blue. Visible mycelium was then transferred on sterilized petridish containing SDA (Sabouraud's Dextrose Agar medium; Agar- 20 gms, Dextrose- 40gms, peptone- 10 gms, distilled water-1000 ml) for the isolation of fungi.

Purification and Identification of Keratinophilic fungi

Desirable colonies appearing on SDA medium were subcultured till the pure culture of the fungi was obtained. After the purification of the culture the keratinophilic

fungi was identified with the help of the literature available in the Laboratory manual of Introductory Mycology (Alexopaulus and Benedek 1962), Manual of soil fungi (Gillman, 1957), Manual of Chrysosporium and Allied genera (Van-oorchoot 1980) and A colour Atlas of Pathogenic fungi (Frey *et. al.* 1986).

Results and discussion

In the present investigation, 120 soil samples were analyzed 60 samples from each site. It was observed that out of 60 samples of both sites 47 were recorded positive for hilly and 34 for plain. The percentage prevalence of fungal species in hilly and plain areas was calculated 78.3 % and 56.6 % respectively.

In case of non-keratinophilic fungi species only four species were isolated i.e. *Aspergillus niger, Mucor* sps, *Rhizopus* sps and *Fusarium* sps of which all of them were recorded in hilly areas whereas only *Aspergillus niger* and *Mucor* sps were isolated from plain areas. The species isolated from hilly areas are *Chrysosporium Georgia, C. keratinophilum, C. lobatum, C. sulfureum, Geomyces pannoru m, Microsporum audouinii, M. boullardii, M. fulvum, Trichophyton ajelloi, T. saudiense and Zymonema* sps. The species isolated from plain areas are *C. queenslandicum, Cladosporium* sps, *Geomyces pannorum, M. boullardii, Emmonsia parva, M. gypseum, M. vanbreuseghem, T. mentagrophytes, T. simii and T. yaundiae.*

Total five species each of genera *Chrysosporium, Trichophyton, Microsporum* and one species each of *Cladosporium, Emmonsia, Geomyces* and *Zymonema* were isolated from both the areas. The average frequency for *Microsporum fulvum* maximum, which was found in 7 out of 40 samples with percentage prevalence of 14.8% while in case of plain area *Trichophyton simii* was dominant colonizer with percentage prevalence of 17.6 % as shown in fig. 1. The most of the species of genera *Chrysosporium, Microsporum* was dominant in hilly areas while lacking in plain areas. Out of the five species each of *Chrysosporium, Microsporum* and *Trichophyton* only one species of *Chrysosporium* and three species each of the remaining two genera were found in plain areas.

Among the genera *Chrysosporium, C. Georgia* was dominant colonizer in hilly areas (10.6%) followed by *C. keratinophilum* (8.5%), *C. lobatum* (8.5%) and *C. sulfureum* (6.3%) while lacking *C. queenslandicum* that was present in plain with 11.7% frequency and lacking the remaining three species of *Chrysosporium.*

The dominant species of *Microsporum* in hilly areas were *Microsporum fulvum* (14.8%) followed by *M. boullardii* (6.3%) and *M. audouinii* (4.2%). In contrary among the species of *Microsporum, M. gypseum, M. vanbreuseghem* was found to be first and second order of incidence with 14.7% and 11.7% distribution respectively in plain

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areas followed by *M. boullardii* with percentage prevalence of 5.8%.

Trichophyton species isolated from baits amended with the soil of hilly areas were dominantly colonized by *T. saudiense* (8.5%) followed by *T. ajelloi* (6.3%) while plain areas were encountered maximally by *Trichophyton simii* (17.6%) and *T. yaundiae* (11.7%) and least by *T. mentagrophytes* (2.9%).

Cladosporium sps and *Emmonsia parva* were distributed with 5.8% in plain areas while completely absent in hilly areas and *Zymonema* sps shows the vice-versa condition with 4.2% prevalence. *Geomyces pannorum* and *Microsporum boullardii* were the only keratinophylic fungi found in both the sites with moderate percentage prevalence.

Chryosporium sps was the most abundantly found in hilly areas with four species namely C. *Georgia, C. keratinophilum, C. lobatum and C. sulfureum* with abundance 31.2%, 25%, 25% and 18.7% respectively. Only one species of *Chryosporium* i.e. *C. queenslandicum* was abundantly found in plain areas with 100% frequency. Overall *Chrysosporium* genera show diversified nature in hilly areas in comparison to plain areas.

Microsporum shows similar presence in both the areas irrespective of presence of species. The three species of *Microsporum* i.e. *Microsporum audouinii, M. boullardii* and *M. fulvum* were present in hilly areas with abundance i.e. 58.3%, 25% and 16.6% whereas in plains *M. gypseum, M. vanbreuseghem* and *M. boullardii* was abundantly found with frequency of 45.4%, 36.3% and 18.1%. Only *M. boullardii* was present in both the sites showing its capacity to perpetuate well in extreme conditions. Among the five species of *Trichophyton* two species were found in the hilly areas while three were isolated from plain areas. *T. ajelloi* and *T. saudiense* with abundance 42.8 and 57.1% respectively was found in hilly areas while *T. mentagrophytes, T. simii* and *T. yaundiae* were isolated from plain areas with abundance 54.5%, 9.09%, 36.3% respectively.

Table 1: Keratinophilic and non-keratinophilic fungi isolated from hilly and plain areas

Name of isolates	Hilly area (Shimla, H.P.)			Plain area (Agra, U.P.)		
	Occurrence In no. of Samples	Frequency (%)	Adundance (%)	Occurrence In no. of Samples	Adundance (%)	Frequency (%)
Total no. of positive sample	47/60	78.3		34/60	56.6	
Non-keratinophilic fungi						
Fusarium sps	2	28.5	100	0	0	0
Aspergillus niger	3	42.8	100	1	50	100
Mucor	1	14.2	10	1	50	100

Rhizopus	1	14.2	100	0	0	0
Keratinophilic fungi	5	10.6	31.2	0	0	0
	4	8.5	25.0	0	0	0
Chrysosporium georgiae	4	8.5	25	0	0	0
C. keratinophilum	3	6.3	18.7	0	0	0
C. lobatum	0	0	0	4	11.7	100
C. sulfureum	0	0	0	2	5.8	100
C. queenslandicum	0	0	0	2	5.8	100
Cladosporium sps	3	6.3	100	1	2.9	100
Emmonsia parva	2	4.2	16.6	0	0	0
Geomyces pannorum	3	6.3	25	2	5.8	18.1
Microsporum audouinii	7	14.8	58.3	0	0	0
M. boullardii	0	0	0	5	14.7	45.4
M. fulvum	0	0	0	4	11.7	36.3
M. gypseum	3	6.3	42.8	0	0	0
M. vanbreuseghem	0	0	0	1	2.9	9.09
Trichophyton ajelloi	4	8.5	57.1	0	0	0
T. mentagrophytes	0	0	0	6	17.6	54.5
T. saudiense	0	0	0	4	11.7	36.3
T. simii	2	4.2	100	0	0	0
T. yaudiae						
Zymonema sps						

A comparative account in these sites shows that they differ from each other in climatic and other ecological conditions. The hills (Shimla, H. P.) provide a cool climate and soil rich in humus with thick vegetation in contrast to plain sites (Agra, U. P.), which have a dry climate with high temperature during major part of the year along with many other vegetation differences. The percentage of the samples in hilly (Shimla, H.P.) was maximum i.e. 78.3 % (47/60) followed by plain areas {56.6 % (34/60)}. Results obtained for the prevalence of the keratinophilic fungi confirmed the findings of Deshmukh (1982), Govil *et. al.* (2001), Singh *et. al.* (1994) w.r.t the maximum no. of isolated keratinofers in cool climate. Further Garg (1996) in his study has shows the same type of distribution of dermatophytes in the plain areas of Rajasthan and hilly areas of Kashmir. Thakur *et. al.* (1982) have also reported the higher incidence of ringworm infection in goats during November to March. The varied prevalence of keratinophilic fungi in soils of different part of India may be due to some distinction in the climatic conditions (Deshmukh and Agarwal 1998). Singh *et al.* (1990) in a survey with positive growth with a frequency of 67.2 % which is in somehow parallel with a percentage prevalence of keratinophilic fungi i.e. 56.6 % in present study. They found the *Chrysosporium tropicum* with maximum dominance, which contradicts with the findings, of present survey in which the *Trichophyton simii* shows the maximum dominance. Again Singh *et. al.* (1995) found *Microsporum gypseum* as dominance dermatophytes from soils of Agra but in present study *M.*

gypseum occurs with average frequency. Therefore the differential prevalence suggested that the species dominant in one set of circumstances might not necessarily be able to compete equally under different set of circumstances (Ghawana 1997). During the present study, investigation of the soil was done by the hair baiting technique (Vanbreuseghem 1952), which is selective for keratinophilic fungi. Sharma *et. al.* (1997) reported the effectiveness of keratin baiting technique is the detection of a broad spectrum of fungi in biological waste and compost. The keratinophilic fungi such as *Microsporum* speices, *Trichophyton* species isolated in this study are characteristics of this group. Soil surveys conducted in Australia reported similar type of fungi for e.g. *Chrysosporum, Cladosporium, Fusarium, Microsporum, Trichophyton, Mucor* and *Verticillium* species (Soo-Hoo 1991, Marchisio *et. al.* 1991). In a similar study by Ghawana *et. al.* (1997) analysed a woolen baits for more than one-year indicating the fungal colonization was initiated by the non keratinophilic fungi (Saprophytic fungi) which grew luxuriously on the baits and utilized as sole source of nutrient, at least some part of various non-keratinised intercellular structures such as medullary trichohyaline and soft keratin might undergo digestion. Kumar *et. al,* 2012 reported the isolation of keratinophilic fungi in which maximum percentage of *Aspergillus niger* (12.06%) and *Fusarium oxysporum* (10.34%) followed by other fungal genera was found in the Piggery soil of Jharkhand, India. In a study by Singh *et al.*, 2014, various species of keratinophilic fungal genera i.e. *Acremonium, Chrysosporium, Microsporum, Malbranchea* and *Trichophyton* were isolated from the soil of Dharamshala, Himachal Pradesh. Pakshir *et. al.* 2013 isolated 22 genera of keratinophilic fungi from the soil of public parks in Shiraz, Iran of which *Fusarium* (23.8%) & *Acremonium* (12.65%), was reported to be present in maximum amount following other species too. According to the work of Sharma & Choudhary, 2015, out of twenty two soil samples eight different fungi, 4 species belongs to *Trichophyton* and *Microsporum* each were isolated using a spread plate method. In the present work, maximum percentage of *Microsporum fulvum* in plain area (Agra) and *Trichophyton simii* in hilly areas (Shimla) were found reportedly.

Conclusion

It was evident from the results that keratinophilic fungi and allied dermatophytes survive saprophytically on soil. Keratin a natural complex proteinaceous substrates is decomposed by the keratinolytic activity of these fungi. Various ecological factors affect and control their survival, distribution, pathogenesis and keratinolytic activity. The biotechnological importance of the present work was the isolation and screening of the keratinophilic fungi.

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