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SOME KERATINOPHILIC FUNGI AND RELATED DERMATOPHYTES FROM SOIL

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Soil samples collected from different localities frequented particularly by various animals and birds were baited for the isolation of fungi capable for colonizing and attacking keratinous substrates. In all 94 fungi were isolated. Distribution of keratinophilic fungi in various types of soils and on different kind of keratinic substrates are discussed. Some fungal species new to Indian fungal flora have been also mentioned.

INTRODUCTION

Keratinophilic fungi are potentially pathogenic to man and animals and includes dermatophytes. Studies in soil inhabiting keratinophilic fungi from various habitats are receiving considerable attention in recent years. The significance of ecology of keratinophilic fungi with special reference to dermatophytes have become the subject of enquiry by a large number of workers. Several reports are there in the literature showing geophilic dermato—and keratinophytes (Pugh and Mathison 1962; Kishimoto and Baker 1969; Ajello and Alpert 1972).

So far as India is concerned, studies of this kind are rather fragmentary and very limited. Dey and Kakoti (1955) were the first to report, *Microsporum gypseum* (Bodin) Gujart and Grigorakis from soil samples of Dibrugarh, Assam. Later reports of isolation of the fungi of the same nature were also made by Randhawa *et al.* (1959), Padhye (1961), Puri (1961), Padhye and Thirumalachar (1962), Randhawa and Sandhu (1965), Garg (1966), Padhye *et al.* (1967). In the present study an effort was made to determine the prevalence of keratinophilic fungi from soil samples collected from various sources. Substrate relationship of the isolates was also determined and discussed.

MATERIALS AND METHODS

Samples of soil and decomposing keratinous substances were collected in sterilized polythene bags from different localities of Sagar, and its suburban areas. Samples were taken from the superficial soil layer, depth not exceeding 2-3 inches. These samples were transported to the Mycological laboratory and were processed immediately or kept at

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15°C temperature until processed. In the collection of soil samples preference was given to the areas which were frequently inhabited by animals or birds. Few samples from garden and crop field soils were also taken. In addition to these, animal house floor sweepings and dropped off feathers were also used for isolation purpose. Isolations were also made from decomposing keratinous substances like horns, hair, hooves, nails, feathers and wool.

Isolation by baiting

Five to eight grammes of soil from each sample was taken in Petridish and moistened with 10–20 ml of sterile distilled water. These were baited by burying various keratinic substances in soil. The following keratinrich substances were used as baits.

Feather—Feathers of various birds were made into pieces of 1–2 inches, boiled in distilled water for about 15–20 min and used as baits.

Hair—Human hair were cut into pieces of 2–4 cm, autoclaved and used as baits. Besides human hair pig, buffalo, cow and horse hair were also used in the same way.

Horn—Cow and buffalo horns were used, these were broken into small pieces, sterilized for 15 min at 15 lb pressure and then buried in soil.

Nail—Human nails were collected and sterilized. A few were deeply inserted in soil samples and some were kept directly on moist soil aseptically.

Wool—Pure wool pieces were taken, sterilized in distilled water at 15 lb pressure for 10 min and used as baits.

Direct isolation from keratinic substances

(A) Pieces of decomposing keratinic substances (feather, hair, horn, nail, etc.) were collected and brought to the laboratory, after removing soil particles gently these were placed in moist chamber, after 4–6 days the molds growing on these samples were transferred directly in Petridishes having suitable medium.

(B) The dropped off feathers and other keratinic substances were collected and incubated in moist chamber at 28 °C for 8 –10 days. Periodical observations were made under binocular microscope for mold growth and to get sporulation. The samples were examined and the portions where the fungal spores were observed were marked and immediately the spores were picked up aseptically under the micromanipulator then transferred to fresh dishes having Sabouraud's dextorse agar medium. The dishes were incubated at 28°C and after complete growth the purity of cultures was confirmed.

The fungi appeared on different type of baits and from different sources are given in Table I and Figs. 1–2.

Results and Discussion

A total of 94 fungi were isolated. These isolates represent 60 species among 23 genera and 7 sterile forms. Forty-four fungal species were found on buried animal horns Table I. In fungal colonization next to horn was hair showing 22 species of various genera. Ten species were isolated from buried nails and 11 species from degrading wool pieces. The number of molds on feather was comparatively low and confined to seven only.

TABLE I

Colonisation of keratinophilic fungi on different substrates

Organisms	Baits used				
	Horn	Hair	Nail	Feather	Wool
Cunninghamella elegans	—	—	—	+	—
Synecephalastrum racemosum ITCC 1876	—	+	—	+	—
Chaetomium indicum ITCC 1871	—	+	—	—	—
C. globosum KIMI 179869	+	—	—	—	—
Keratinophyton terreum IMI 185313	+	—	—	—	—
Aspergillus nidulans	+	—	—	—	—
Acrodontium albaeo IMI 179837	—	+	+	—	—
Aspergillus fumigatus	+	+	+	—	—
A. niger	+	+	—	—	+
A. quercinus IMI 179848	—	—	+	—	—
A sachhari	+	—	—	—	—
A. sulphureus	+	—	—	—	—
A. ustus IMI 179847	+	+	—	—	+
Botryotrichum keratinophilum IMI 185322	—	+	—	—	—
B. piluliferum	+	+	—	—	—
Chrysosporium crassetunicatum IMI 185320	+	+	—	—	—
C. tropicum strain 1 IMI 179840	+	+	+	+	+
C. tropicum strain 2 IMI 179839	+	—	—	—	—
C. tropicum strain 3 IMI 179838	+	—	—	—	—
C. tropicum strain 4 IMI 179836	—	+	—	—	—
Cephalosporium acremonium ITCC 1877	+	—	—	—	—
Cladosporium chlorocephalum	—	+	—	—	—
Curvularia indica ITCC 1879	+	—	—	—	—
C. geniculata	+	—	—	—	—
C. subulata	+	—	+	—	—
Fusariella concinna ITCC 1891	+	—	—	—	—
Fusarium sp.	+	—	—	—	—
F. brachygibbosum	+	—	—	—	—
F. chlamydosporum	+	—	—	—	—
F. nivale	+	—	—	+	—
F. oxysporum	+	—	—	—	—
F. sporotrichoides	+	—	—	—	—
F. poe	+	—	—	—	—
F. roseum	—	+	+	—	—
Gliocladium sp. IMI 179846	+	—	—	—	—
G. deliquescence	+	—	—	—	+
G. roseum IMI 179845	+	—	—	—	—
G. penicilloides ITCC 1887	—	—	—	—	+
Helminthosporium sativum	—	—	+	—	—
Humicola fusco-atra ITCC 1873	—	+	—	—	—
Malbranchea pulchella IMI 179843	+	—	+	—	—
Microsporum fulvum IMI 194744	—	+	—	—	—
Microsporum gypseum IMI 194745	+	+	+	+	+
Penicillium auraunti bruneum	—	—	—	—	+
P. duclauxi	+	—	+	—	—
P. lilacinum	+	—	—	—	—
P. rubrum	—	—	—	—	+
Paecilomyces fusisporus	+	+	—	—	—
P. varioti ITCC 1893	+	—	—	—	—
Trichoderma harzianum IMI 179833	+	—	—	—	—
T. hematum IMI 185323	+	+	—	—	—
T. viride	+	—	—	—	—

TABLE I (*Contd.*)

Organisms	Baits used				
	Horn	Hair	Nail	Feather	Wool
Trichophyton mentagrophytes IMI 194747	+	+	+	+	+
T. rubrum IMI 194746	—	+	—	—	—
Verticillium sp. IMI 179841	—	—	—	+	—
V. lecanii IMI 185318	—	—	—	—	+
Sterile 1 IMI 185313	+	—	—	—	—
Sterile 2 IMI 185315	+	+	—	—	—
Sterile 3 IMI 185316	—	—	—	—	+
Sterile 4 IMI 185317	+	—	—	—	—
Sterile 5 IMI 185319	—	+	—	—	—
Sterile 6 IMI 185321	+	—	—	—	—
Sterile 7 IMI 179834	+	—	—	—	—

+Sign indicates presence, —Sign indicates absence.

There was a noticeably higher yield of keratinophilic fungi from forest litter samples Fig. 1 corelates with higher influx of rabbits, birds and other forest animals which include their remains in soil. The well-known dermatophytes i.e., *Microsporum* spp. *Trichophyton* spp., some species of *Aspergillus* and *Penicillium* were isolated from samples of animal house floor sweepings, cattle and poultry farms. The data showed that very restricted number of fungal species could colonise buried feathers, although these were showing strong keratinolytic ability when tested by the method described by Agrawal and Kushwaha (1974). It is well established that numerous fungi pathogeni c to human and animals are discriminated by birds and insects (Pugh 1964-65). The samples from crop fields yielded only seven fungal species. *Acrodontium* was restricted to forest litter soils only. Keratinophilic strains of Aspergilli were recorded in most of the samples except dropped off feathers. *Chaetomium* spp. were restricted to forest litter soils only and *Botryotrichum* to the samples of garden, forest and cattle farms. Species of *Chrysosporium, Trichophyton* and *Microsporum* seem to be higher in distribution. Phycomycetous genus *Syncephalastrum* was found in samples collected from crop field and cattle farms, while the other genus of this class, i.e., *Cunninghamella* was isolated from soils of poultry farms only.

It is interesting to note that species of *Acrodontium, Cladosporium* and *Humicola* were found colonizing hair only in the same way *Cephalosporium, Fusariella* and *Keratinophyton* were recorded from decomposing horn pieces only. Phycomycetous genus *Cunninghamella* was recorded only on decomposing feathers. This may be due to the preferential nutrition of fungi as well as hardness and also on the availability of the exposed surface of various keratinized substrates used as baits. The results presented here add to the increasing evidence that the choice of nutrition requirements may vary in species of the same genus.

Some of the isolates repoited here are well-known dermatophytes causing superficial infeciions of keratinized tissue (Emmons *et al.* 1963) viz., *Trichophyton mentagrophytes, Trichophyton rubrum* and *Microsporum gypseum.* Ajello (1960) categorized dermatophytes as anthropophilic, zoophilic and geophilic. According to him except *Trichophyton mentagrophytes* other species of *Trichophyton* are mostly

anthropophilic or zoophilic. In this study *T. mentagrophytes* has been isolated only from soil showing its geophilic tendencies. The same pathogen has also been recorded as geophilic by Padhye *et al.* (1966) from soils of Madras, Ajello and Cheng (1967); Kishimoto and Baker (1969) from soils of Hawaii. Padhye *et al.* (1966) recorded this pathogen from soil samples of poultry farms, cattle farms and animal house floor sweepings. *Trichophyton rubrum* and *Microsporum gypseum* were described by Frey (1965) Padhye *et al.* (1966) Kishimoto and Baker (1969) as animal pathogens, these were of common occurrence during isolation.

A total of seven species of *Aspergillus* i.e., *A. flavus, A. niger, A. quercinus, A. sacchari, A. sulphureus, A. ustus* and *A. nidulans* were recorded on different baits. *A. quercinus* was encountered on human nails only. A potentially pathogenic strain of *A. niger* was reported by Kishimoto and Baker (1969). They also recognised *A. fumigatus* as pathogenic one. Keratinolytic ability of some of these isolates was also succesfully confirmed by the method described by Agrawal and Kushwaha (1974).

Penicillium lilacinum and *P. rubrum* could colonize only horn and wool pieces respectively. *T. hematum* and *T. viride* were isolated from horn pieces. Pugh (1966) in his study also reported *T. viride* on birds and Griffin (1960) isolated *T. viride* from buried hair. *Chrysosporium tropicum* a widely known keratinophilic fungus was encountered frequently during isolations. It has been also reported from soils by Garg (1965) and Padhye *et al.* (1966).

Curvularia subulata, C. geniculata and *C. indica* were isolated from hair pieces. This forms the first report of these *Curvularia* species as keratinolytic. Nityanand *et al.* (1962) reported mycotic keratitis caused by *C. lunata.* The same fungus was also described by Pugh (1966) from birds.

Some keratinophilic species of *Cephalosporium, Cladosporium* and *Fusarium* were also isolated using different baits. Several species of these genera viz., *Cephalosporium acremonium, Cladosporium chlorocephalum* and *C. werenkii* have been already reported as dermatophytie (English, 1965; Pawai *et al.* 1965; Kishimoto and Baker 1969). The keratinophilic fungi like *Fusarium oxysporum* and *F. solani* were reported to cause keratitis and subcutaneous lesion by Zimmerman (1962) and English (1965) respectively. A species of *Humicola,* i.e., *H. fusco-atra* was found to colonize hair. *H. grisea* was reported by Griffin (1960) to colonize keratinized substances. *H. fusco-atra* has been reported here for the first time as keratinolytic.

Four ascomycetous fungi i.e., *Aspergillus nidulans, Chaetomium indicum, C. globosum* and *Keratinophyton terreum* were isolated from different keratinic substances. Several species of *Chaetomium* including *C. indicum* were also isolated by English (1963) from nail samples.

Among the Phycomycetes, *Cunninghamella elegans* and *Syncephalastrum racemosum* were isolated during this work. Two representatives of this group have been recognized as pathogenic by Kishimoto and Baker (1969). *S. racemosum* was found to be associated with ear, nail or corneal infections in man (English 1965). She also isolated *C. elegans* from keratinized material. In addition to above described fungal species seven sterile forms were also collected from decomposing keratinic substances. No sporulation was observed in these isolates even after treatments of ultravoilet light and in a variety of nutritions. These isolates showed the keratinolytic activity when grown on various types of wool and hair.

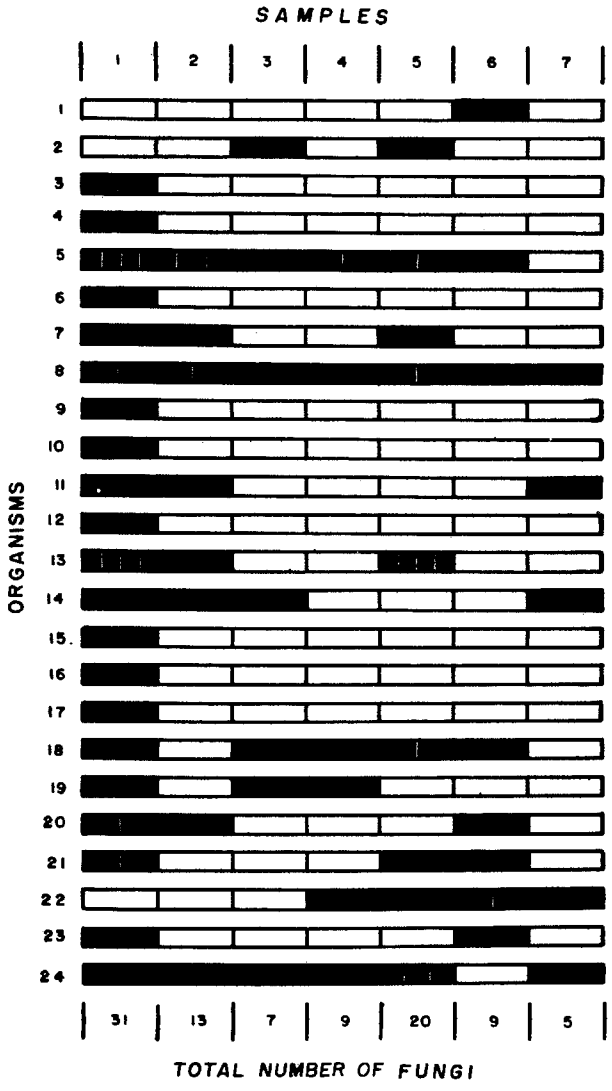

FIG. 1. Keratinophilic fungi as recorded from various samples. Samples: 1, Forest litter; 2, Garden soil; 3, Crop field soil; 4, Animal house-floor sweepings; 5, Cattle farms soil; 6, Poultry farms soil; 7, Dropped off feathers.

Organisms: 1, *Cunninghamella*; 2, *Syncephalastrum;* 3, *Chaetomium;* 4, *Keratinophyton;* 5, *Aspergillus;* 6, *Acrodontium;* 7, *Botryotrichum;* 8. *Chrysosporium;* 9, *Cephalosporium;* 10, *Cladosporium;* 11, *Curvularia;* 12, *Fusariella;* 13, *Fusarium;* 14, *Gliocladium;* 15, *Helminthosporium;* 16, *Humicola*; 17, *Malbranchea;* 18, *Microsporum;* 19, *Paecilomyces;* 20, *Penicillium;* 21, *Trichoderma;* 22, *Trichophyton;* 23, *Verticillium;* 24, Sterile mycelium.

(*Note*: Black bar shows presence of the organism and breaks in bar show number of isolates)

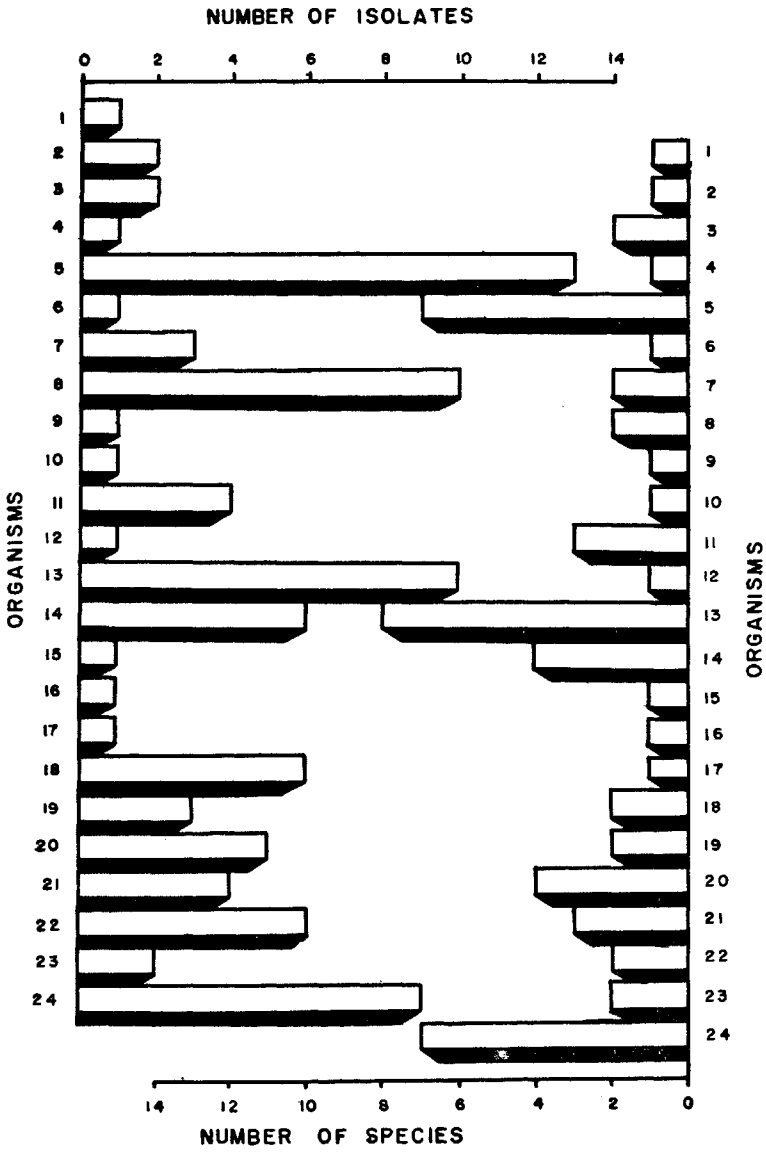

FIG. 2 Fungi representing species and their isolates.

Organisms: 1, *Cunninghamella;* 2, *Syncephalastrum;* 3, *Chaetomium;* 4, *Keratinophyton;* 5, *Aspergillus;* 6, *Acrodontium;* 7, *Botryotrichum;* 8, *Chrysosporium;* 9, *Cephalosphorium;* 10, *Cladosporium;* 11, *Curvularia;* 12, *Fusariella;* 13, *Fusarium;* 14, *Gliocladium;* 15, *Helminthosporium;* 16, *Humicola;* 17, *Malbranchea;* 18, *Microsporum;* 19, *Paecelomyces;* 20, *Penicillium;* 21, *Trichoderma;* 22, *Trichophyton;* 23, *Verticillium,* 24, Sterile mycelium.

The genus *Acrodontium* and *Malbranchea* has not been repoıted previously from Indian soils. Therefore their isolations from soil and their keratinophilic tendencies are particularly noteworthy.

The present study also added several fungal species viz., *Verticillium* sp., *V. lecanii* and *Trichoderma hematum* which were not previously known from India and noted here as keratinophilic.

All the fungi discussed above are keratinophilic in nature and a few of them are well known pathogens causing skin diseases of man and also agents of deep mycoses. If these etiologic agents are present in an active state even for a short period, they can serve as potential foci of infection for man and animals inhabiting these localities. Theii occurrence in the soil is of epidemiological importance. The further work on the physiology and the nutritional study of these isolates is in progress.

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