J. Mycopathol, Res, **50(2)** : 193-198 (2012)

Keratinophilic fungi: their occurrence in the environment

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Received : 20.10.2011 Accepted : 22.05. 2012 Published : 29.10.2012

Keratin occurs in nature in the form of hair, fur, feathers, wool, horns, hooves, nails, claws and cornified appendages of birds and animals. The localized accumulation of the keratin wastes cause a serious disposal problem leading to environmental pollution. The industrial disposal methods have ecological disadvantages in terms of energy expenditure. Recently, an alternative method of using microorganisms in degrading the keratinic wastes has been devised. A special group of keratin degrading fungi (keratinophilic fungi) present abundantly in the soil produce an enzyme keratinase which is used for de-hairing in leather industries. Besides, it is also used in the production of protein rich feather meal for poultry, slow nitrogen releasing fertilizer, enzyme based detergents, biodegradable films, coatings, glue, vaccines for dermatophytosis and additives in skin lightening agents. Hence there is an urgent need to isolate and conserve the keratinophilic fungi for exploitation of their potential.

Key words : Keratin, keratinophilic fungi, occurence, soil

INTRODUCTION

Keratin is mechanically hard and chemically unreactive due to its numerous cross-links of disulphide bonds, which stabilize the stereo-chemical configurations of this scleroprotein (Alexander and Hudson, 1954). The distinctive feature of keratin is its relatively high sulphur content due to the presence of sulphur containing aminoacids cysteine, cystine and methionine. A few organisms like insects, bacteria, actinomycetes and fungi are able to breakdown and utilize keratin as the sole source of carbon and nitrogen. Several fungi have the unique ability to colonize keratinous substances and degrade them. They are called 'keratinophilic fungi'. These 'keratin loving' organisms are the minute machines that cycle one of the most abundant and highly stable animal proteins on earth (Kushwaha and Gupta, 2008). They have many properties in common with the dermatophytes and hence every keratinophilic fungi can be considered as a potential pathogen (Rippon, 1982).

Soils that are rich in keratinous substrates are the reservoir of keratinophilic fungi (Chmel *et al.* 1972). Forests, farmyards, parks, burrows, slaughter houses, feather and hair dumping sites, fertile soils, sewage sludge, sediments of rivers and oceans containing rich humus and organic matter are the best sites for the growth of these fungi (Ali- Shtayeh and Rana, 2000).

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ISOLATION OF KERATINOPHILIC FUNGI

The keratinophilic fungi can be isolated from the soil by two major techniques. Hair baiting technique (HBT) (Vanbreuseghem, 1952) and surface soil dilution plating technique (SSDP) (Stanghellini and Hancock, 1970). The hair baiting technique is the most common method used for the quantitative and qualitative isolation of keratinophilic fungi from the soil whereas the latter technique is only used for the quantitative isolation. The keratinophilic fungi isolated by these techniques can be purified and identified by microscopic studies in lacto.phenol cotton blue stain, with reference to standard mycology manuals.

FERTILE LAND

Chrysosporium carmichaelii, C. georgii, C. indicum. C. keratinophilum, C. lobatum, C. merdarium, C.

OCCURRENCE OF KERATINOPHILIC FUNGI

Chrysosporium sp.,	*Microsporum* sp.,	*Trichophyton* sp.,	Others	Reference
C.keratinophilum	*M.fulvum* *M gypseum*	*T.ajelloi,* *T.terrestre*	*Arthroderma quadrifidum,* *Nannizzia gypsea*	*Rogers, (1971)*
C.asperatum, *C.evolceanui* *C.pruinosum,* *C.tropicum*	*M.gypseum*	*T.ajelloi*	*Auxarthron thaxteri,* *Chaetomium murorum,* *C.spirale, Ctenomyces serratus,* *Gymnoascus petalosporus,* *G.reesii, Malbranchea* state of *Ch. uncinatus*	*Guarro et al. (1981)*
C.keratinophilum, *C.tropicum*	*M.audouinii* *M.canis* *M.fulvum,* *M.gypseum,* *M.vanbreuseghemi*	*T.ajelloi,* *T.mentagrophytes,* *T.soudanenese,* *T. yaoundel*	*Curvularia lunata*	*Ogbonna* and Pugh, (1987)
C.asperatum, *C.carmichaelii,* *C.lucknwense,* *C.pannicola,* *C.pruinosum,* *C.xerophilum*	*M.gypseum*	*T.equinum,* *T.mentagrophytes,* *T.rubrum,* *T.soudanenese*	*Alternaria alternata, A. raphani,* *A.tenuissima, Aphanoascus* sp., *Apinisa queenslandica,* *Arthroderma lenticulare,* *A.cuniculi, A. curreyi,* *Aspergillus flavus,* *A.fulvescens, A. fumigatus,* *A.terreus, A.ustus, A. wentii,* *Cunninghamella elegans,* *Fusarium oxysporum,* *Macrophomina phaseolina,* *Penicillium chrysogenum* *P. citrinum, P. funiculosum,* *P.puberulum,* *Pectinotreichum llanense,* *Rhizopus stolonifer,* *Ulocladium chartarums*	El-Said, (1995)
C.indicum, *C.keratinophilum,* *C.lobatum,* *C.pannicola,* *C.tropicum,* *C.* st of *Arthroderma cuniculi, C.* st of *Ctenomyces serratus*	*M.fulva,* *M.gypseum*	*T.mentragrophytes,* *T.terrestre*	*Arthroderma simii.* *Ctenomyces serratus,* *Gymnascella hyalinospora,* *Malbranchea aurantiaca.* *Pseudogymnoascus roseus*	Deshmukh, (2002)
--	*M.canis,* *M.gypseum*		*Aspergillus candidus, A.flavus,* *A.fumigatus, A. nidulans,* *A.niger, A.ustus, A.wentii,* *Cunninghamella echinulata,* *C.elegans*	*Irum et al.* (2007)
C.indicum, *C.tropicum,* *C.zonatum*	*M.gypseum*	*T.mentagrophytes*	*Aphanoascus fulvuscence,* *Aphanoascus punsolae,* *Spiromastix warcupii*	Deshmukh *et al.* (2008)

pannicola, C. queenslandicum, C. tropicum, C. anamorph *of Arthroderma cuniculli, C.* anamorph of *Arthroderma curreyi, C.* anamorph of *Pechtinotrichum illiase, C.* anamorph of *Rollandina vriesii* were isolated from the water logged paddy fields (Shrivastava *et al.* 2008).

SWIMMING POOLS

Trichophyton mentagrophytes occur commonly in the floors of swimming pools (Khaleel. 1999), swimmers and swimming pools (English and Gibson, 1959; Detandt and Nolard, 1995). Species of *Alternaria, Aspergillus, Cladosporium, Fusarium, Geotrichum, Penicillium* and *Phoma* were commonly isolated from swimming pools (Aho and Him, 1981; Maghazy *et al.,* 1989). *Acremonium strictum, Aspergillus flavus, Chrysosporium keratinophilum. C. merdarium, C tropicum, Cladosporium cladosporioides, Geotricum candidum, Gliocladium nigrovirens, Paecilomyces lilacinus. Penicillium chrysogenum* and *Trichophyton terrestre* were reported from the swimming pools, polluted and unpolluted stream sites (Ali-Shtayeh *et al.*,2000).

WATER SEDIMENTS

Chrysosporium evolceanui, C. keratinophilum, C. tropicum, Microsporum gypseum and *Trichophyton terrestre* were reported from a small pool (Mangiarotti and Caretta, 1984). *Acremonium implicatum, Chrysosporium indicum, C. georgii, C. keratinophilum, C. xerophilum, Geomyces pannorum. Malbranchea* sp. and *Microsporum gypseum* were reported for the first time from the water sediments in India (Katiyar and Kushwaha, 2002).

COASTAL AREAS

Salt pans are very common in the coastal habitats. Seawater flows into the salt pans bringing keratinic materials during high tides. Several keratinophilic fungi *viz, Acremonium sclerotigenum. Acrophialophora fusispora, Allescheria boydii, Arachniotus dankaliensis. Aspergillus flavus, Cephalosporium acremonium, C. indicum, C. keratinophilum, C. tropicum, Fusarium moniliforme, Gymnoascus uncinatus. Humicola grisea, Malbranchea* state of *Penicillium funiculosum. Microsporum gypseum, Mucor hiemalis, Myrothecium verrucaria, Scopulariopsis brevicaulis, Verticillum intertextum* (Abdel-Fattah *et al.* 1982) and *Chrysosporium fluviale, C. indicum, C. tropicum, C. zonatum, C.* state of *Ctenomyces serratus, Ctenomyces serratus, Malbranchea aurantiaca, Microsporum gypseum, Trichophyton mentagrophytes, T. terrestre, Uncinocarpus reesii* were isolated from the coastal areas (Deshmukh, 1999 ; 2004).

LONAR LAKE

Aphanoascus durus, A. punsolae, Auxarthron kuehnii, Chrysosporium indicum, C. tropicum, C. state of *Ctenomyces serratus* were collected from the vicinity of Lonar lake, a meteorite crater in India (Deshmukh and Verekar, 2006).

GLACIER BANKS

Chrysosporium keratinophilum, C. tropicum, C. state of *Ctenomyces serratus, Geomyces pannorum. Malbranchea* sp., *Microsporum gypseum, M. nanum, M. vanbreuseghemii. Trichophyton ajelloi, T. terrestre* and *Uncinocarpus reesii* were reported from glacier banks of Kashmir valley (Deshmukh, 2002).

SEWAGE SLUDGE

The distribution of keratinophilic fungi in the waste waters have been studied by various investigators (Park, 1972; Abdel-Hafez and El-Sharouny, 1990; Abdullah and Hassan. 1995; Ali-Shtayeh *et al.* 1999). *Chrysosporium keratinophilum* with *Aphanoascus keratinophilus. Microsporum gypseum* with *Arthroderma* sp., *T. terrestre* with its teleomorph *Arthroderma quadrifidum* and *T. ajelloi* with *A. uncinatum* (Ulfig 1986; Ulfig *et al.* 1997; 1998; Ali-Shtayeh *et al.*;1999), and *Geotrichum* sp.. prevailed in the sewage sludge (Hedayati and Mirzakhani, 2009).

AIR

Several filamentous fungi including *Chrysosporium indicum, Geomyces pannorum* var. *pannorum, Microsporum gypseum, Myceliopthora vellerea* and *Trichophyton terrestre* were reported from Pavia, Italy (Delia Franca and Caretta, 1984).

SCHOOLS

Keratinophilic fungi from the floor dusts of the classrooms revealed the presence of the species belonging to the genus *Chrysosporium. Microsporum* and *Trichophyton* (Mercantini *et al.* 1983; Ali-Shtayeh and Arda, 1989; Ramesh and Hilda, 1999) and *Epidermophyton floccosum* (Mercantini *et al.*,1986).

PARKS

Parks are a common place of visit by human, animals and hence rich in keratinous residues. The most common species like *Chrysosporium asperatum, C. keratinophilum, C. pannorum* (Shadzi *et al.* 2002), *C. tropicum*, C. state of *Arthroderma tuberculatum*, C. state of *Ctenomyces serratus*, *M. cookei, Trichophyton ajelloi* and *T. terrestre* (Marsella and Mercantini, 1986), *C. indicum, C. keratinophilum, C. luteum, Microsporum cookie, M. gypseum, Trichophyton ajelloi*, and *T.terrestre* (Papini *et al.* 1998) were screened from the soil of city parks.

INDOOR POTTED PLANTS

Species of *Aphanoascus, Arthroderma, Botryotrichum, Malbranchea, Microsporum, Trichophyton* and *Verticillium* were isolated from soil of planted earthen pots in the indoor environments (Singh *et al.,* 2009 a).

HOSPITALS

A few keratinophilic dermatophytes and related species viz, *Chrysosporium keratinophilum, C. tropicum, Microsporum gypseum, M. nanum. Trichophyton mentagrophytes* (Vidyasagar et al., 2005; Singh *et al.*, 2009 b). *C. keratinophilum. C. pannorum. C. tropicum. Chrysosporium* state of *Arthroderma tuberculatum, M. canis. M. gypseum. T. ajelloi, T. mentagrophytes and T. terrestre* (Mancianti and Papini. L396) prevailed the floors and corridor dusts of private clinics.

POULTRY FARMS AND FEATHER DUMPING SITES

Chrysosporium keratinophilum. C. state of *Arthroderma tuberculatum. Geomyces pannorum, Microsporum gypseum, Myceliopthora vellerea. Trichophyton mentagrophytes* were recovered from the soils of poultry farms (Anbu *et al.,* 2004). Species of *Aspergillus, Acremonium, Alternaria. Beauvaria, Curvularia, Paecilomyces, Penicillium* (Marcondes *et al.*, 2007) including *Aspergillus niger, A. flavus. A. fumigatus. A.wentii, Botrytis cineria. Chochliobolus lunatus. Chrysosporum asperatum. Fusarium* sp.,*Mucor* sp., *and Penicillium* sp., were reported from poultry farm wastes (Soomro *et al.* 2007; Ganaie *et al.* 2010).

BIRDS

Keratinolytic fungi like *Anixiopsis stercoraria, Arthroderma ciferrii, A. curreyi, A. tuberculatum, A. quadrifidum. Chrysosporium keratinophilum, C. tropicum, and Ctenomyces serratus* were recovered from the nests of free-living birds (Hubalek *et al.*, 1973). Species of *Aspergillus, Chrysosporium, Fusarium, Microsporum, Mucor, Penicillium, Rhizopus, Trichophyton* were isolated from feathers, nails and beaks of 20 common birds including chicken, ducks, turkeys and pigeons (Efuntoye and Fashanu. 2001) and *Aspergillus, Chaetomium, Chrysoporium, Curvularia, Fusarium, Malbranchea, Microascus, Penicillium, Sepedonium, Scopulariopsis* from feathers of living poultry birds (Kaul and Sumbali, 1999).

ANIMALS

Microsporum canis, M. gypseum, Trichophyton ajelloi, T. terrestre (Ali-Shtayeh *et al.*, 1988; Elizabeth *et al.*, 2000), *M. nanum* (Ali-Shtayeh *et al.*;1988), *C. keratinophilum, C. tropicum. Trichophyton mentagrophytes* and *T. terrestre* (Mancianti *et al.*, 1997) and *T. verrucosum* (Elizabeth *et al.*, 2000) were isolated from the skin of animals.

ANIMAL HERDS

Soil samples from animal herds (camels, goats, sheep, and cows) were surveyed for the occurrence of keratinophilic fungi. Eleven *Chrysosporium* species were reported in the following order of dominance: *C. carmichaelii, C. indicum, C. keratinophilum, C. pannicola, C. queenslandicum,* C. state of *Arthroderma curreyi,* C. state of *A. cuniculi* and *Chrysosporium* state of *Renispora flavissima*, *Aphanoascus* teleomorph of *C. keratinophilum, C. tropicum and C, zonatum* (Al-Musallam, 1990).

HEAD AND SCALP OF HUMAN

Alternaria alternate, Aphanoascus fulvescence, *Arthroderma cuniculi, Aspergillus flavus. Cladosporium cladosporioides, Cl. herbarum, Chrysosporium asperatum, C. carmichaelii, C. farinicola, C georgii, C keratinophilum, C. merdarium, C. pannicola, C. pannorum. C. pseudomerdarium, C.queenslandicum , C. sulfureum, C. tropicum , C. xerophilum, Gymnoascus demonbreanii, Microsporum canis, M. nanum, Penicillium chrysogenum. Trichophyton violaceum, T. concentricum, T. tonsurans . T. violaceum* and *Verticillium albo-atrum* were reported from the head and scalp mycobiota of clinically normal children of age 6-12 (Ali-Shtayeh *et al.* 2000).

FACTORS AFFECTING THE GROWTH OF KERATINOPHILIC FUNGI

The distribution of dermatophytes and keratinophilic fungi depend on ecological, physical and chemical factors particularly pH, dissolved oxygen concentrations, temperature, heavy metals, and organic matter especially keratinaceous substances (Chmel *et al.,* 1972). A possible relationship exists between the keratinophilic fungi and the environment (Ali-Shtayeh *et al.* 2000). The isolation of *Chrysosporium* sp., (Pugh and Mathison, 1962) and *Microsporum gypseum* (Bohme and Ziegler, 1969; Chmel *et al.* ,1972) from coastal soil might be due to the favorable pH of those soils, the availability of nutrients, climate conditions and the enrichment of the soils by the molted feathers of birds and fish debris, that favour aleurosporic fungi. The distribution and survival of keratinophilic fungi may also be influenced by the ecological conditions, in addition to the availability of the keratinous substrates in the soil. Soils with pH ranging from 6 to 9 were observed to be best suitable for luxuriant growth and highest frequency of keratinophilic fungi (Bohme and Ziegler, 1969). Certain keratinophilic fungi in soil were found to be selective with high (5%) or low (1.5%) humus content (Ajello and Padhye 1974; Abdel-Fattah *et al.,* 1982; Al-Musallam, 1989).

CONCLUSION

The degradative enzyme keratinase produced by the keratinophilic fungi are used widely in various industries. The enzymatic bio-conversion of vast quantities of the keratinic wastes produced from the poultry farms to useful products such as protein fodder offers a cheap and easy method for the production of value-added products. Hence there is a growing need to isolate and screen the non-dermatophytic fungi from the environment for their industrial applications.

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