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ISOLATION AND CHARACTERIZATION OF KERATINOPHILIC FUNGI AND RELATED DERMATOPHYTES FROM VARIOUS PUBLIC PARKS OF JAIPUR, INDIA

SUBHASH GORA*, MAMATA GORA, NEETU JAIN, SHAMSHAD UL HAQ, MEENAKSHI SHARMA, SUNITA MAHARIYA,

Medical Mycology and Microbiology Laboratory, Department of Botany, University of Rajasthan, Jaipur, Rajasthan-302004, India

ABSTRACT

Keratinophilic fungi and related dermatophytes are important both economically as well as naturally because they play an important role in degradation of keratin material present in soil, which is due to human and animal presence. They grow mostly on keratin protein's decomposition product, obtained from decomposition of hair, nails, horns, claws etc. Present study characterized soil samples from 20 public parks of Jaipur district, India for occurrence of keratinophilic fungi. A total of 15 species belonging to 5 genera were isolated and identified from 125 soil samples. 79 soil samples were found positive, from which 144 isolates of keratinophilic fungal were recovered. *Chrysosporium tropicum* being the most prevailing of all with 22 isolates and present in 12 public parks out of 20 (60 %), followed by *C. indicum* and *Trichophyton mentagrophytes*. *Epidermophyton* was least spread of all, present only in 4 parks, out of 20 public parks. In many positive samples more than 2 isolates were obtained. *Microsporum canis* prevalence in public parks is the finding of this study which is due to roaming of rouge animals.

KEYWORDS: **keratinophilic fungi, dermatophytes, public parks, soil, *Trichophyton.***

SUBHASH GORA

Medical Mycology and Microbiology Laboratory, Department of Botany, University of Rajasthan, Jaipur, Rajasthan-302004, India.

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INTRODUCTION

Keratin protein is among one of the most non-degradable protein on earth surface degraded only by an important group of fungi called keratinophilic fungi, due to which it gets degrade and circulate back to environment. Keratinophilic fungi are dominated in both natural and man-made environment. Their occurrence is directly correlated with keratinaceous materials (like hair, nails, claws, furs, horns etc.) present in soil environment, which is shredded by humans and animals. Soil is the most important supporting medium for keratinophilic fungi perpetuation, which latter on act as potential source of infection for human and animals[1,49]. Soil rich in keratinaceous materials are most suitable for the growth of keratinophilic fungi. Saprophytic fungi and keratinophilic fungi are mostly found in humus and organic materials which found in park soil, farmyard soil, sediments of river and oceans[2]. Raghukumar[51] has done extensive survey of marine coast especially around Maharashtra coast and west coasts of India. Mercantini *et al*[3] stated that human and animal's dominance at a particular environment enhances keratinophilic fungus' presence. Keratinophilic fungi presence in soil is quantified by various author at different places like Abdul-Hafez *et al* (Egypt)[4],Al-Musallam (Kuwait)[5], Ali-Shtayeh (Palestine)[6], Calvo *et al* (Spain)[7], Soon (Malaysia)[8], Deshmukh (India)[28], Marchisio *et al* (Italy)[9]. Above all studies indicates that keratinophilic fungi show worldwide occurrence in soil. Keratinaceous materials are common in soil because it is not degraded by normal microbial succession. These materials are only degraded by substrate specific fungi called keratinophilic fungi that colonize keratinaceous substrate like hairs, nails, claws, horns etc[10]. Other microorganisms found associated with keratinophilic fungi can only enhance the degradation process. Supporting microorganisms are dependent upon degraded product left over by keratinophilic fungi[11]. Fungal succession is not so simple and linear as it is presented in plant and animal community. In fungal succession actual replacement of fungal mycelium is not needed[12]. Rayner and Todd[38] clearly stated that fungal succession is just occupation of same site by different fungal mycelium or various associations of fungi. A broad study about how a particular fungus pioneer a virgin place, which later on get replaced primarily and secondarily by another fungus, due to any environmental disturbance is conducted by Rayner and Boddy[13], Wicklow[14], Pugh and Boddy[15] and Goyal *et al*[16]. There are two groups which are keratin dependent in nature, one is keratinolytic fungi and another is keratinophilic fungi[17]. Distinction between them depends upon the way they utilize keratin protein. Keratinolytic fungi directly depend upon keratinaceous materials present in soil and decomposed it completely and are highly pathogenic to humans and animals[18]. While keratinophilic fungi is dependent upon material associated with keratin or resulting from its destruction[19]. Human and animal mycoses is due to one of group of keratinophilic fungi called dermatophytes that's why it is widely studied in the field of medical and veterinary sciences [20]. Majority of dermatophytes belongs to these three genera- *Epidermophyton, Microsporum* and *Trichophyton*. All these genera are anamorphic in nature. They are restricted to non-living cornified layer because they are unable to penetrate viable tissue below cornified layer[50]. They are generally classified on the basis of conidial morphology and how conidia get formed (classification given originally by Emmons[21], which later on get updated by Matsumoto and Ajello[22]). Keratin protein is mainly present in hair, feathers, wool, nails, horns etc., which is degraded by keratinase enzyme[23]. Keratinophilic fungus is solely dependent upon keratin protein, both as a source of carbon and nitrogen, but their nitrogen content is low (8-14 %) as compared to other microorganism due to higher rate of deamination. Hedayati *et al*[24] and Ramesh and Hilda[25] studied presence of Keratinophilic and dermatophytic fungi in soil. Their study clearly states that soil is a good source of keratinaceous materials for the growth of keratinophilic fungi. Keratinophilic fungi present in every environment condition, with variable distribution pattern, directly dependent upon human and animal presence. In India various investigator conducted a broad study from various habitats viz. poultry farm (Shukia *et al*[26]), water sediments (Katiyar and Kushwaha[27]), glacier bank (Deshmukh[28]), birds and their environment (Sur and Ghosh[29],Jain and Sharma[33]) primary schools (Ramesh and Hilda 1998)[25], lake side soil (Ghosh and Bhatt[30]), hilly area (Deshmukh[28], Bhadauria and Kushwaha[31], Deshmukh and Verekar[32]), salt pans (Deshmukh[34]), indoor dust (Singh *et al*[35]), aquatic sediments (Gupta and Kushwaha[52]) etc. Most important factor for growth of keratinophilic fungi is temperature and relative humidity which is favorable during the month of February and August. Growth of dermatophytes are also encouraged or enhanced by hot and humid conditions, which directly correlate with tropical and temperate regions of world[36]. Current study in relation to garden soil is more important because garden and park are highly crowded by humans and pet animals, which leads to increase in keratinaceous materials in soil and hence increase in keratinophilic fungi. As stated earlier that keratinophilic fungi are potent pathogen against animal and human being so a garden soil survey of keratinophilic fungi will be of hygienic and epidemiological importance. In addition a broad survey will also lead to discovery of various dermatophytes around us in our garden/parks will leads to general awareness in people.

MATERIALS AND METHODS

Collection of Soil Samples

Soil samples were collected from February 2015 to April 2015 in sterilized polythene bags (10x20 cm). A total of 122 samples were collected from 20 municipality park (name given in table I) of Jaipur district. Nearly on an average 5 samples were collected from each park for accurate sample size. Sample size varies according to number of person visit the park i.e. more sample were collected from park which are more crowded. 20 municipality parks were located in various locality of Jaipur district and maintained by Jaipur Development Authority. Before sampling, upper layer of large size debris containing soil were removed (up to depth of 1 to 1.5 cm) with the help of sterilized spatula. From each site a sample of approximately 400 gm is collected. These samples are collected and sealed in plastic bag

and taken to laboratory situated at Department of Botany, University of Rajasthan, Jaipur. Average time in between sample collection and inoculation in Petri plate is 24 to 36 hours so that saprophytic fungal growth is at minimum.

Hair baiting of soil samples

For the isolation of keratinophilic fungus To-Ka-Va hair baiting method[32] is used. In hair baiting method Petri plate is half filled with soil sample collected from various park and then poured with sterilized distilled water to moist the soil. Then sterilized defatted human hairs are kept on moistened soil surface. These Petri plates are kept under controlled temperature of 25^{o} to 30^{o} C. Each Petri plate is closely examined using binocular microscope every 5th day up to 30 days' time limit.

Isolation, purification and identification of various fungi

When sufficient growth is seen in Petri plate, inoculums are transferred to Sabouraud's dextrose agar medium incubated at 25- 30^{o}C for 7 - 8 weeks aerobically. Medium is added with chloramphenicol (16 µg/mL) and cycloheximide (0.5 µg/mL) to check the growth of bacteria and saprophytic fungi. These fungal growths are then examined microscopically to differentiate various fungal spores. For genera identification slide culture method is used (Bailey and Scott[39]). For fungal identification colour and texture of colony, and pigmentation on the reverse side of colony are the main character.

RESULTS

Out of total 125 samples, 79 samples found positive with63.20 % prevalence of keratinophillic fungi (Table I).

These 125 samples were collected from 20 gardens and parks of Jaipur district, India. Out of 79 samples a total of 144 isolates of keratinophilic fungus were recovered. Among the entire gardens, Deer park situated in the heart of Jaipur city showed highest dominance (100 %) for keratinophilic fungi. All five soil samples collected from Deer park showed growth of keratinophilic fungi. This park is maintained by Government of Rajasthan for deer conservation. 100 % prevalence of keratinophilic fungi in deer park is due to overcrowded communities of deer's and birds in the park. Next to Deer park, the park which showed highest dominance for keratinophilic fungi are Dwarka Das Garden, Dashera Adarsh Park, Chitrakoot Park, Saraswati Garden and Vivekanda Garden. All these parks showed 80 % prevalence of keratinophilic fungi. Among all the 20 gardens, Jawahar Circle Garden showed least dominance (20 %) of keratinophilic fungi. In present study out of 79 positive samples a total of 144 isolates of keratinophilic fungi and related dermatophytes were reported, distributed in 15 species belonging to 5 genera viz. 5 species of *Trichophyton*, 4 species of *Chrsosporium*, 3 species of *Microsporum*, 2 species of *Fusarium* and 1 species of *Epidermophyton* (table II). Out of 15 species *Chrysosporium tropicum* was found to be most dominant of all keratinophilic fungi, with 22 isolates, followed by *C. indicum* (15) and *Trichophyton mentagrophytes* (15). In many positive samples more than 2 isolates were obtained (showed in image C). *Epidermophyton* was least spread, present only in 4 parks. *Microsporum canis* normally dominant in animal habitats were present in 6 parks, highest isolates were from Deer park (Table II).

Table I

Soil samples from different Public Gardens/Parks of Jaipur City.

S.No.	Garden Name/Site	No. of Sample taken	Total +ve samples	% Occurrence
1.	Nehru BalUdhyan	5	3	60.00
2.	Central Park	12	5	41.66
3.	Deer Park	5	5	100
4.	KulishSmriti Van	9	7	77.77
5.	Jawahar Circle Garaden	10	2	20.00
6.	KalpanaChawla Park	3	2	66.66
7.	Sh. Bhagat Singh park	5	3	60.00
8.	Ran Nivas Garden	10	7	70.00
9.	KanakVrindavan Garden	10	6	60.00
10.	Dwarka Das Garden	5	4	80.00
11.	Chandra Shekhar Garden	4	3	75.00
12.	DasheraAadarshMaidan	5	4	80.00
13.	Vivekananda Garden	4	2	50.00
14.	Gurunanak Park	3	1	33.33
15.	Ashok Vatika	3	2	66.66
16.	Nehru Park	5	3	60.00
17.	JDA Mansrowar Park	7	4	57.14
18.	Chitrakoot Park	5	4	80.00
19.	Saraswati Park	5	4	80.00
20.	Vivekananda Park UOR	10	8	80.00
	Total Sample	125	79	63.20

Table II
Distribution of individual fungal species in different habitats with number of isolates.

Fungi / Public Parks	Total no. of sample	No. of +ve sample	*C.tropicum*	*C. indicum*	*T.mentagrophytes*	*M. audouinii*	*T. simii*	*T. rubrum*	*M. gypseum*	*C. evolcunai*	*C. keratinophilum*	*F.oxyspourm*	*F. moniliformae*	*Epidermophyton spp.*	*T. verrucosum*	*M. canis*	*T. terrestre*
	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	N
Nehru Bal Udhyan	5	3	2	-	1	-	-	-	1	-	-	1	-	-	-	1	-
Central Park	12	5	3	-	-	-	1	1	1	-	-	-	1	-	-	-	-
Deer Park	5	5	1	-	1	-	-	-	2	1	-	1	-	-	-	2	-
Kulish Smriti Van	9	7	2	1	2	-	1	-	2	-	1	-	-	-	-	2	1
Jawahar Circle Garden	10	2	-	3	-	1	-	1	-	-	1	-	1	1	-	-	-
Kalpana Chawla Park	3	2	-	1	1	-	-	-	-	1	-	-	1	-	1	-	-
Sh. Bhagat Singh park	5	3	1	-	-	-	1	-	-	1	-	-	-	-	-	-	1
Ran Nivas Garden	10	7	3	2	2	-	-	1	1	-	1	-	-	1	-	2	-
KanakVrindavan Garden	10	6	-	-	3	-	2	1	-	1	-	-	1	-	1	-	-
Dwarka Das Garden	5	4	-	2	-	1	1	-	-	-	1	-	1	-	1	-	1
Chandra Shekhar Garden	4	3	-	1	1	-	-	1	1	-	-	-	-	1	-	-	-
Dashera Aadarsh Park	5	4	1	-	-	1	-	1	-	-	1	-	1	-	-	1	-
Vivekananda Garden	4	2	-	1	-	1	-	-	1	-	-	1	-	-	1	1	-
Gurunanak Park	3	1	1	-	1	-	-	-	1	-	-	1	1	-	-	-	-
Ashok Vatika	3	2	1	1	1	-	-	-	-	-	1	-	-	-	1	-	-
Nehru Park	5	3	-	1	-	1	-	1	-	-	2	-	1	-	-	1	-
JDA Mansrowar Park	7	4	2	-	-	1	-	-	1	1	-	1	-	1	-	-	1
Chitrakoot Park	5	4	1	-	2	-	1	-	1	-	1	1	-	-	1	-	-
Saraswati Park	5	4	-	2	-	-	1	-	-	-	-	1	-	-	-	1	-
Vivekananda Park UOR	10	8	4	-	-	1	-	-	2	-	-	1	-	-	-	1	1
No. of total isolates of each species			22	15	15	7	8	7	14	5	9	8	8	4	5	12	5

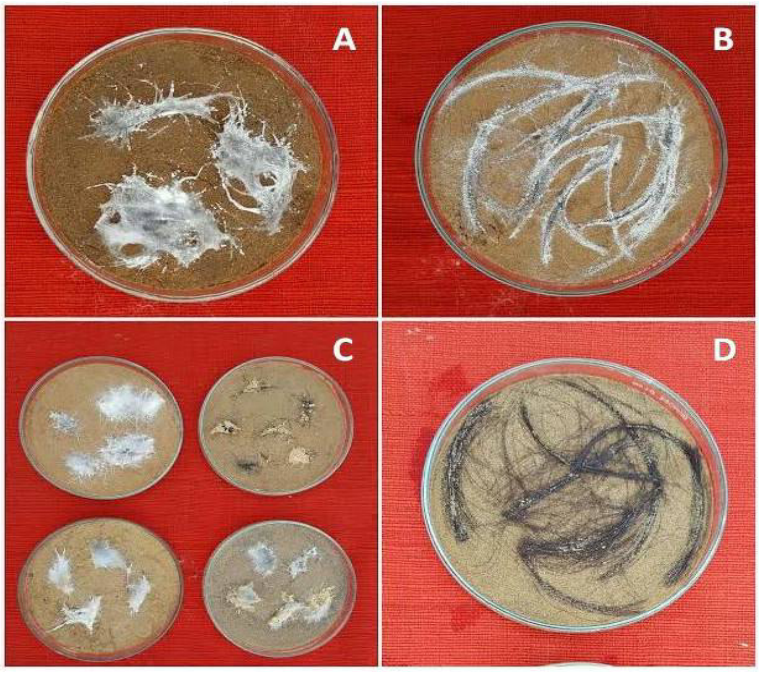

Figure I
Petri plate is showing single isolate of keratinophilic fungus (A) and (B). Mixed growth of different keratinophilc fungus in single soil sample (C). No growth of fungus (D).

DISCUSSION

Keratinophilic fungus plays a major role in degradation of keratinized material present in soil[40-41]. However, this study is quite important because no one had surveyed public parks soil alone, for the occurrence of keratinophilic fungi flora. Therefore, a total of 125 were collected from 20 public park of Jaipur city for the evaluation of dermatophytic and keratinophilic fungi. 79 samples found positive for the keratinophilic fungus, which yield 144 isolates. Study revealed that *Chrysosporium tropicum* is most dominant, followed by *Microsporum gypseum* and *Chrysosporium indicum.* Jain and Sharma[33] done a wide survey of soil from University of Rajasthan campus and found that *Chrysosporium tropicum* (20.83%) was the most predominant fungi followed by *Trichophyton mentagrophytes* (15.10%). Jain and sharma[44] also had done similar study from various sites of Jaipur, India and isolated *Trichophyton verrucosum, Microsporum audouinii* and *M. canis* for the first time in Jaipur. In this study *Chrysosporium tropicum* (46.08 %) was the most predominant fungus followed by *Trichophyton mentagrophytes* (24.88 %). Ramesh and Hilda[25] found similar result for primary school and public park of Madras city. They found 31 species of keratinophilic fungi belonging to 15 genera, among which *Chrysosporium tropicum* (62.2 %) is the most dominated, followed by *C. keratinophilum* (48.8 %) and *M. gypseum* (48.8 %). Mahariya *et al*[42] surveyed soil samples from different site of Jaipur, India and found that *Chrysosporium tropicum* and *Trichophyton rubrum* are the most dominant of all. Similarly in a study from garden soil of Mumbai, India, following species were isolated viz. *Chrysosporium indicum, Microsporum gypseum, Trichophyton mentagrophytes* and *Uncinocarpus reesii*[32]. Garden soil survey from Tunisia evaluated that *Chrysosporium keratinophilum* was the major species (30.5 %) followed by *Microsporum gypseum* (27.4 %). Shukia *et al*[26] studied *Microsporum gypseum* from soil sample of various poultry farm from Rewa, Madhya Pradesh and concluded that out of 37 samples, 25 were positive for the *Microsporum gypseum*. Study on hilly area of Himachal Pradesh showed that there are 11 species of five genera among which prominent keratinophilic fungus are viz. *Chrysosporium tropicum, C. indicum, C. queenslandicum, C. keratinophilum, C. xerophilum, Microsporum gypseum, M. canis, Ctenomyces serratus, Malbranchea gypsea* and *Trichophyton mentagrophytes*[32]. Saxena *et al*[45] took 284 soil samples from cattle yards, crop fields, hospitals, poultry farms and playgrounds from Agra, India and found that 204 samples were positive for the keratinophilic fungi, among which *Sporotrichum* spp. is the most prominent followed by *Trichophyton simii.* Rekha and Krishnaveni[46] surveyed soil sample and water sample for *Microsporum* spp. dominance from South Tamilnadu and isolated five species of *Micropsorum* viz. *M. gypseum, M. canis, M. nanum, M. distortum and M. cookei.* Gugnani *et al*[47] conducted similar study on keratinophilic fungi at Jamaica, finding 75 % sample positive for keratinophilic fungus occurrence with 50 % dominance of *Micrsporum gypseum.* Shadzi *et al*[48] collected 330 soil samples from elementary schools and seven public parks in the province of Isfahan, Iran and isolated seven species of keratinophilic fungi among which most frequent isolate was *Chrysosporium keratinophilum* (54.2%). A study was conducted on sandpits in Turin, Italy in which 57 species were isolated with nearly 52 % prevalence of keratinophilic fungi. Species of *Microsporum, Aphanoascus, Chrysosporium, Geomyces, Trichophyton, Mariannaea* and *Malbranchea* are the major ones[9].

CONCLUSION

Present study concluded that keratinophilic fungi and related dermatophytes are in high number in soil, so chance of acquiring infection from soil is high, when we walk bare footed on public parks soil. Another major finding is *Microsporum canis* from garden soil of Ram Nivas Garden, Dashera Adarsh Park and Vivekananda Park, which is mostly confined to animals in public parks. So a better monitoring of public parks by municipality must be there to evade rouge animals.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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Reviewers of this article

Dr. Seema Bhadauria, Ph.D.

Head & Associate Professor, Department of Microbiology, JECRC University, Jaipur, India

Prof. Y. Prapurna Chandra Rao

Assistant Proffessor, KLE University, Belgaum, Karnataka

Prof.Dr.K.Suriaprabha

Asst. Editor , International Journal of Pharma and Bio sciences.

Prof.P.Muthuprasanna

Managing Editor , International Journal of Pharma and Bio sciences.

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