Prevalence of keratinophilic fungi in public park soils of Mumbai, India

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Abstract

The parks of Mumbai are frequently visited by local residents every morning and evening. However, there are no reports on the occurrence of keratinophilic fungi in these areas. The purpose of this research was to study the occurrence of keratinophilic fungi in the public parks of Mumbai. One hundred soil samples were collected from five public parks: Kamla Nehru Park, Powai Garden, CD Deshmukh Garden, Five Gardens and Chota Kashmir. Keratinophilic fungi were isolated by the hair baiting technique using human hair as keratin bait. The cultures were identified using macro- and micro-morphological features. Identification was also confirmed by the BLAST search of sequences of the ITS1-5.8S-ITS2 rDNA region against the NCBI/Genbank data and compared with deposited sequences. The ability of these fungi to use human hair was also evaluated by release of protein in liquid media. A total of 75 strains of keratinophilic fungi were recovered from 100 (75.0%) soil samples. The isolated fungi were composed of eleven species of eight genera: *Arthrographis kalrae*, *Auxarthron conjugatum*, *Chrysosporium indicum*, *C. queenslandicum*, *C. zonatum*, *Gymnascella dankaliensis*, *G. hyalinosporea*, *Microsporum gypseum* (15.0%), *Myriodontium keratinophilum*, *Trichophyton mentagrophytes* and *Uncinocarpus reesii*. These fungi can release 148.8-307.6 µg/mL protein in liquid media when grown on human hair in shake flask culture and also decompose 16.2-38.6% of human hair after four weeks of incubation. Our study indicates that keratinophilic fungi are to be found in the soils of various public parks in Mumbai and that human hair can be a source of pathogenic fungi.

Introduction

Keratinophilic fungi are an ecologically important group of fungi that decompose one of the most abundant and highly stable animal proteins on earth, keratin, which they use as a nutrient substrate for growth. The distribution of these fungi depends on different factors,

including the vitally important human and/or animal presence.¹ Some of these fungi are well-known dermatophytes and are known to cause superficial cutaneous infections (dermatophytoses) of keratinized tissues (skin, hair and nails) of humans and animals. Mycotic infection is reported throughout the world and is extremely contagious.² The occurrence of dermatophytes in soil was reported for the first time by Vanbreuseghem³ using the hair bait technique. Since then, studies on the isolation of keratinophilic fungi from soil have been investigated throughout the world.⁴⁻⁸

Mumbai (18°58'N, 73°51'E) is the financial capital of India with a population of about 10 million. It has a tropical climate with a high level of humidity. Its coastal and tropical location ensures moderate temperatures throughout the year, with an average temperature of 27.2°C and average precipitation of 242.2 cm (95.35 inches).⁹ This type of climate favors the growth of fungi. There have been some reports from Mumbai of dermatophytic infections.¹⁰⁻¹¹ In previous studies, we have attempted to examine whether soils of salt pan, beaches and caves are a reservoir of these fungi.¹²⁻¹⁴

The public parks in Mumbai are frequently visited by local residents. Some areas of these parks are used by children as a playground. Some residents bring their pets to the park. These parks are also often invaded by animals such as cows, bullocks, pigs, cats and rats. Local birds are common to these parks: sparrow (*Passer domesticus*), pigeon (*Columba livia domestica*), dove (*Streptopelia risoria*), crow (*Corvus brachyrhynchos*), parrot (*Psittacula krameri*) and myna (*Acridotheres tristis*). Human activity may contribute to the keratinic material to be found in the soil. Transit animals leave organic residues which may contaminate the soil with keratinous debris and can provide reservoirs for fungi. It would, therefore, be important to analyze and identify the mycoflora of public parks in order to evaluate the extent of the diffusion of keratinophilic fungi in these environments and the resultant health risk.

Materials and Methods

One hundred soil samples were collected from five public parks in Mumbai: Kamla Nehru Park, Powai Garden, CD Deshmukh Garden, Five Gardens and Chota Kashmir from March 2008 to February 2009 (Table 1). The samples were collected from the superficial layer of soil at a depth not exceeding 3-5 cm and placed in sterile polyethylene bags, brought to the laboratory, and stored at 15°C for a maximum of two weeks if not processed promptly. Soil from the gardens is mostly

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manure and litter, and soil pH varied from 6.5 to 8.5. Half decomposed feathers and hair (both from human and animal origin) were also found in garden soil.

Keratinophilic fungi were isolated by the hair baiting technique of Vanbreuseghem³ using human hair as keratin bait. For this purpose, sterile petri dishes half-filled with the soil samples and moistened with sterile water were baited by burying sterile human hair in the soil. These dishes were incubated at room temperature (28±1°C) and examined daily from day 5 for fungal growth over a period of four weeks. After observing the growth under a stereoscopic binocular microscope, isolates were cultured on Sabouraud's dextrose agar supplemented with chloramphenicol (50mg/L) and cycloheximide (500 mg/L).

Identification of these fungi was based on the monographs of Sigler and Carmichael,¹⁵ Oorschot,¹⁶ Currah,¹⁷ Arx von,¹⁸ and Cano and Guarro¹⁹ using macro- and micro-morphological features.

Molecular characteristics of the cultures were studied by determination of their DNA

sequences of the ITS1-5.8S-ITS2 region. Genomic DNA was extracted by the miniprep protocol of Lee and Taylor.²⁰ The ITS1-5.8S-ITS2 rDNA was amplified using primers ITS1 and ITS4 as the forward and reverse primers, respectively, as described by White *et al.*²¹ and followed by Deshmukh and Verekar.⁶ The final products were analyzed by electrophoresis on 1.2% agarose (Sigma). The PCR products were purified using Qiagen Gel extraction kit (CAT No. 28704) and the PCR products of expected size were sequenced using ITS1 and ITS4 primers in an Applied Biosystem 3730 DNA analyzer at MWG, Bangalore, India. A BLAST²² search was made of the sequences against the NCBI/Genbank data and compared with deposited sequences for identification purposes.

Substrate decomposition

The substrate decomposition and protein released into the medium from human hair was monitored using the procedures set out by Lowry *et al.*,²³ and Deshmukh and Agrawal.²⁴ Protein determinations from filtrates were carried out after four weeks of incubation. The developing color was read at 660 nm on a Shimadzu UV-VIS spectrophotometer. Freshly prepared human albumin serum was used as the standard. The results of protein determinations were expressed as net values, *i.e.* the measured value in the test sample minus the sum of values of keratin and fungus controls of 11 isolates (Table 1). Experimental values are expressed as mg of protein per mL of supernatant. All the experiments were carried out in triplicate at 28 $\pm$ 1 $^{\circ}$ C with appropriate controls. The rate of hair decomposition was determined using the method of Chester and Mathison.²⁵

Results and Discussion

The results of the isolations are presented in Table 1. They show that out of 100 samples only 75 yielded keratinophilic fungi which were categorized into 11 species of 8 genera: *Arthrographis kalrae* (3.0%), *Auxarthron conjugatum* (2.0%), *Chrysosporium indicum* (26.0%), *C. queenslandicum* (6.0%), *C. zonatum* (5.0%), *Gymnascella dankaliensis* (7.0%), *G. hyalinosporea* (3.0%), *Microsporium gypseum* (15.0%), *Myriodontium keratinophilum* (1.0%), *Trichophyton mentagrophytes* (4.0%) and *Uncinocarpus reesii* (3.0%).

All the eleven strains yielded unique PCR amplification. The sequences of the ITS1-5.8S-ITS2 rDNA region for the eleven strains were from 528 bp to 680 bp. *Myriodontium keratinophilum* and *Trichophyton mentagrophytes* were the smallest and the largest, respectively. The other species showed a product size of approximately 600 bp. There was considerable difference in the sequence data of the eleven strains analyzed. The data were also compared with sequences deposited in the NCBI/Genbank for identification purposes. The ITS data of the isolated strains were identical to the ITS data of *Arthrographis kalrae* (AB116536.1), *Auxarthron conjugatum* (AJ608967.1), *Chrysosporium indicum* (AJ005369.1), *C. queenslandicum* (AB219228.1), *C. zonatum* (AB219229.1), *Gymnascella dankaliensis* (AY304514), *G. hyalinosporea* (AF129853.1), *Microsporium gypseum* (JN134130.1), *Myriodontium keratinophilum* (EU925387.1), *Trichophyton mentagrophytes* (HQ014707.1) and *Uncinocarpus reesii* (AB361653.1).

Chrysosporium indicum (26.0%) was found to be the most dominating species followed by

M. gypseum. *Chrysosporium indicum* was reported from India's plains in various studies and dominates Indian soil mycobiota because it is adapted to warmer conditions.^{7,26-28} *Microsporium gypseum*, a well-known geophilic dermatophyte, had shown a frequency of 15.0%, it has also been reported from Indian plains.²⁹⁻³¹ *Gymnascella dankaliensis* frequency followed that of *M. gypseum* at 7.0. Another species of *Gymnascella* was *G. hyalinosporea* (3.0%). Both these cultures were reported from soils of Orissa by Roy *et al.*,³² and from Kerala by Deshmukh.³³ *Gymnascella dankaliensis* was also reported from caves around Mumbai, India.¹⁴

Chrysosporium queenslandicum (6.0%) was the next dominating species of *Chrysosporium* after *C. indicum* followed by *C. zonatum* (5.0%). Various species of *Chrysosporium* have been reported from Indian soils.³⁴⁻³⁶ Other species of fungi were *Auxarthron conjugatum* (2.0%) and *Myriodontium keratinophilum* (1.0%). *Auxarthron conjugatum* was reported from India's plains in various studies³⁷⁻³⁸ and *Myriodontium keratinophilum* was reported from pigeon feathers from Maharashtra³⁹ and also from the soils used in mud houses in Khammam, India.⁴⁰

In the present study, we found *Arthrographis kalrae* in 3.0% of soil samples and this is reported in soils from various parts of world.¹⁵ Similarly, *Uncinocarpus reesii* was found in 3.0% of soil samples only; Ajello and Padhye⁴¹ isolated it from the nesting sites of the Galapagos Islands, it has also been isolated from Chilka Lake, which is the largest saline lake in India,⁴² and from salt pan soils of Mumbai.¹² Regarding the dermatophytes, we found *Trichophyton mentagrophytes* besides *M.*

Table 1. Distribution of keratinophilic fungi from public park soil samples, their proteolytic ability and % decomposition of human hair.

Source of soil samples	Kamla Nehru Park	Powai Garden	C D Deshmukh Garden	Five Gardens	Chota Kashmir	Total	% distribution	Protein released (μ g/mL)	% decomposition
No. of samples examined	20	20	20	20	20	100			
No. of samples positive	14	16	15	14	16	75			
Distribution (%)	56	64	60	56	64	75			
<i>Arthrographis kalrae</i>	2	1	-	-	-	3	3	185.8	20.1
<i>Auxarthron conjugatum</i>	-	-	-	2	-	2	2	127.4	16.2
<i>Chrysosporium indicum</i>	7	5	6	3	5	26	26	164.8	24.4
<i>Chrysosporium queenslandicum</i>	2	1	1	1	1	6	6	216.7	27.2
<i>Chrysosporium zonatum</i>	1	2	-	2	-	5	5	246.4	28.2
<i>Gymnascella dankaliensis</i>	-	-	2	3	2	7	7	192.7	22.4
<i>Gymnascella hyalinosporea</i>	-	1	-	-	2	3	3	168.6	20.2
<i>Microsporium gypseum</i> complex	1	3	4	3	4	15	15	288.9	32.6
<i>Myriodontium keratinophilum</i>	-	1	-	-	-	1	1	156.8	18.8
<i>Trichophyton mentagrophytes</i>	1	1	1	-	1	4	4	307.6	38.6
<i>Uncinocarpus reesii</i>	-	1	1	-	1	3	3	148.8	18.8
Total	14	16	15	14	16	75	75		

gypseum in 4.0% of soil samples. *Trichophyton mentagrophytes* has been reported in Indian soils in various studies.^{7,29,30,34,36}

The range of keratinolytic activity was expressed by protein release and percentage of decomposition of human hair. The release of protein ranges from a maximum of 307.6 µg/mL by *T. mentagrophytes* and a minimum of 148.8 µg/mL by *U. reesii* after four weeks of incubation. A similar pattern was observed in percentage of decomposition of human hair. *Trichophyton mentagrophytes* decomposes a maximum of 38.6% of human hair after four weeks of incubation while minimum decomposition was observed in the case of *U. reesii*.

The prevalence of these fungi in garden soils and their keratinolytic activity is of importance for their pathogenic potential and has been confirmed in several investigations in different parts of the world. For example, *Chrysosporium zonatum* was reported to cause disseminated infection in a patient with chronic granulomatous disease.⁴³ In Japan, *C. zonatum* strains were isolated from bronchial lavage from a female in Chiba and from a male in Kyushu. Both patients presented pulmonary cavity sites.⁴⁴ *Chrysosporium tropicum* was reported from comb lesion in two different breeds of chicken in India.⁴⁵ *Neorachnotheca keratinophila* (teleomorph *Myriodontium keratinophilum*) have been reported to cause sinusitis.⁴⁶ There are reports of disseminated infections due to *C. queenslandicum* in garter snakes;⁴⁷ *Gymnascella dankaliensis* was reported from superficial infections in human beings,⁴⁸ and Iwen *et al.*,⁴⁹ isolated *Gymnascella hyalinosporea* from invasive pulmonary infection in a patient with acute myelogenous leukemia. Similarly, Lysková⁵⁰ isolated *Chrysosporium queenslandicum*, *C. sulfureum*, *C. tropicum*, *Malbranchea pulchella*, *Myriodontium keratinophilum* from infections of the skin and nails of patients in the Moravian-Silesian Region (Czech Republic). *Arthrographis kalrae* is a documented etiological agent of mycetoma,⁵¹ photophobia in a contact lens wearer,⁵² sinusitis and meningitis in an AIDS patient,⁵³ sinusitis and ophthalmitis in a healthy individual following trauma to the eye.⁵⁴ Thus, these fungi may be regarded as opportunistic pathogens.

Our study has confirmed that these fungi are to be found in public gardens of Mumbai. On the one hand, they may be playing an important role in decomposing the keratinic matter deposited in these areas but on the other they may be a source of infection to the people visiting the public gardens of this densely populated city.

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