Research Article

Incidence of Keratinophilic Fungi from Selected Soils of Vidarbha Region of Maharashtra State, India

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One hundred and fifty samples were collected from eleven districts of Vidarbha region of Maharashtra state and screened for the presence of keratinophilic fungi using hair baiting technique for isolation. Seventy-one isolates were recovered and identified. The cultures were identified using macro- and micromorphological features. Their 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 for identification purpose. Thirteen species of eight genera were isolated, namely, *Auxarthron conjugatum* (2.00%), *Chrysosporium indicum* (14.00%), *Chrysosporium evolceanui* (2.66%), *Chrysosporium tropicum* (4.66%), *Chrysosporium zonatum* (1.33%), *Chrysosporium* state of *Ctenomyces serratus* (3.33%), *Gymnascella dankaliensis* (1.33%), *Gymnascella hyalinosporea* (0.66%), *Gymnoascoideus petalosporus* (0.66%), *Microsporum gypseum* complex (9.33%), *Trichophyton mentagrophytes* (2.00%), *T. terrestre* (3.33%), and *Uncinocarpus queenslandicus* (2.00%). This study indicates that the soils of Vidarbha region of Maharashtra may be significant reservoirs of certain keratinophilic fungi.

1. 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 utilize as a nutrient substrate for growth. The distribution of these fungi depends on different factors, one of which, of vital importance, is 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 it is extremely contagious [2]. The occurrence of dermatophytes in soil was reported for the first time by Vanbreuseghem [3] using the hair bait technique. Since then studies on the isolation of keratinophilic fungi from soil have been investigated throughout the world [4–7]. Vidarbha is the eastern region of Maharashtra state which lies between 17°57′–21°46′N latitude and 75°57′–80°59′E longitude. The temperature of Vidarbha ranges from a minimum of 12–25°C to a maximum

of 30–48°C with relative humidity varying from 10–15% to 60–95%. Tropical climate and topology of Vidarbha region of Maharashtra make this region a potentially interesting area to study the distribution of these fungi. We therefore undertook this study and report the results obtained.

2. Materials and Methods

2.1. Collection and Processing of Soil Samples. One hundred and fifty samples were collected from various sites of eleven districts, namely, Akola, Amravati, Bhandara, Buldhana, Chandrapur, Gadchiroli, Gondia, Nagpur, Wardha, Yavatmal, and Washim from Vidarbha region of Maharashtra during June 2009–May 2010 (Figures 1 and 2 and Table 1). The samples were collected from the superficial layer of soil at a depth not exceeding 3–5 cm with a plastic spoon in sterile polyethylene bags, brought to the laboratory and stored at 15°C for a maximum of two weeks if not processed promptly. The samples were collected from forest land, cultivated land, road side, public gardens, poultry farm, cattle farm, garbage, and

FIGURE 1: Location of the State of Maharashtra in India.

lake side. For isolation, hair bait technique of Vanbreuseghem [3] was used. Sterile Petri dishes were half filled with the soil samples and moistened with water and baited with keratin by burying sterile human hairs in the soil. Human hairs from a 25-years-old male were used. The hairs were washed several times, first with detergent (hair shampoo), followed by water, and then with diethyl ether, and finally autoclaved at 121°C for 15 min. The dishes were incubated at room temperature (28°C ± 2) and examined daily after 5 days for fungal growth for 4 weeks.

2.2. Isolation and Identification of Keratinophilic Fungi. After observing the growth under a stereoscopic binocular microscope it was cultured on slopes/plates of Sabouraud's dextrose agar supplemented with chloramphenicol (50 mg/L) and cycloheximide (500 mg/L). Cultures were incubated at room temperature for five to ten days following which the cultures were microscopically checked for purity and subcultured to get pure cultures. These fungi were identified based on the various available monographs using macro- and micromorphological features of these cultures [8–12].

2.3. Molecular Identification of Keratinophilic Fungi. 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 [13]. The ITS1-5.8S-ITS2 rDNA was amplified using ITS1 and ITS4 as the forward and reverse primers, respectively, as described by White et al. [14]. Amplification was performed in 100 µL reaction volumes containing 10x buffer 10 µL, MgCl₂ (25 mM) 2 µL, dNTP (10 mM) 2 µL, ITS1

TABLE 1: Distribution of soil samples examined of Vidarbha region of Maharashtra (India).

Districts	Number of samples examined	Number of positive samples	% positive
Akola	24	11	45.83
Amravati	20	9	45.00
Bhandara	15	8	53.33
Buldhana	15	7	46.66
Chandrapur	5	2	40.00
Gadchiroli	5	2	40.00
Gondia	6	4	66.66
Nagpur	15	7	46.66
Wardha	15	5	33.33
Yavatmal	15	8	53.33
Washim	15	8	53.33
Total	150	71	47.33

primer (20 pm) 2 µL, ITS4 primer (20 pm) 2 µL, Taq Polymerase (2.5 U) 1 µL, DNA Sample (5 µg/mL) 3 µL, and Milli Q Water 78 µL. The PCR reaction was carried out using a Thermal Cycler (M.J. research, PTC 200) with conditions as follows: denaturation for five minutes at 94°C, 34 cycles of (30 sec at 94°C, 30 sec at 55°C, and 1 min at 72°C) extension for four minutes at 72°C and storage at 4°C. Negative controls were used in each set of reactions. The final products were analyzed by electrophoresis on 1.2% agarose (Sigma). The PCR products were purified using Qiagen Gel extraction kit (CAT number 28704) and the PCR products of expected size were sequenced using ITS1 and ITS4 primers in an Applied Biosystem 3730 DNA analyzer at GenOmbiotech, Pune, India.

2.4. Phylogenetic Analysis. Similarity analysis of the nucleotides was performed by BLAST searches against sequences available in GenBank [15]. For phylogenetic tree construction, multiple sequences were obtained from GenBank and the alignments were performed using MEGA6 [16].

3. Results and Discussion

The results of the isolations are presented in Table 2. They reveal that out of 150 samples only 71 yielded keratinophilic fungi that can be categorized in thirteen species of eight genera, namely, *Auxarthron conjugatum* (2.00%), *Chrysosporium indicum* (14.00%), *Chrysosporium evolceanui* (2.66%), *Chrysosporium tropicum* (4.66%), *Chrysosporium zonatum* (1.33%), *Chrysosporium* state of *Ctenomyces serratus* (3.33%), *Gymnascella dankaliensis* (1.33%), *Gymnascella hyalinosporea* (0.66%), *Gymnoascoideus petalosporus* (0.66%), *Microsporum gypseum* complex (9.33%), *Trichophyton mentagrophytes* (2.00%), *T. terrestre* (3.33%), and *Uncinocarpus queenslandicus* (2.00%).

TABLE 2: Distribution of keratinophilic fungi in different types of soils of Vidarbha region of Maharashtra.

Source of soil samples	Forest land	Cultivated land	Road side	Public gardens	Poultry farm	Cattle farm	Garbage	Lake side	Total	% distribution
Number of samples examined	20	20	20	25	15	20	20	10	150	
Number of positive samples	6	11	9	13	7	11	9	5	71	
Distribution (%)	30	55	45	52	46.66	55	45	50	47.33	
1 <i>Auxarthron conjugatum</i> (Kuehn) Orr & Kuehn	—	—	1	—	—	1	—	1	3	2.00
2 <i>Chrysosporium indicum</i> (Randhawa & Sandhu) Garg	2	2	3	5	2	4	2	1	21	14.00
3 <i>Chrysosporium evolecanui</i> (Randhawa & Sandhu) Garg	—	1	1	—	—	—	1	1	4	2.66
4 <i>Chrysosporium tropicum</i> Carmichael	—	1	1	2	—	1	1	1	7	4.66
5 <i>Chrysosporium zonatum</i> <i>Al-Musallam & Tan</i>	—	1	—	—	—	1	—	—	2	1.33
6 <i>Chrysosporium</i> state of <i>Ctenomyces serratus</i> Eidam	1	3	1	—	—	—	—	—	5	3.33
7 <i>Gymnoscella dankaliensis</i>	1	—	—	—	—	—	1	—	2	1.33
8 <i>Gymnascella hyalinospora</i> (Kuehn et al.) Currah	—	—	—	1	—	—	—	—	1	0.66
9 <i>Gymnoascoideus petalosporus</i> Orr, Roy & Ghosh	—	—	—	1	—	—	—	—	1	0.66
10 <i>Microsporium gypseum</i> (E. Bodin) Guiart & Grigoraki	1	1	—	3	3	3	2	1	14	9.33
11 <i>Trichophyton mentagrophytes</i> (C.P. Robin) Sabour.	—	—	1	—	1	1	—	—	3	2.00
<i>Trichophyton terrestre</i>	1	—	1	—	1	—	2	—	5	3.33
12 Durie & Frey	—	2	—	1	—	—	—	—	3	2.00
13 <i>Uncinocarpus queenslandicus</i> (Apinis & Rees) Sigler	—	11	9	13	7	11	9	5	71	
Total	6	11	9	13	7	11	9	5	71

FIGURE 2: Sites of collection of soil samples in Vidarbha region of Maharashtra.

All thirteen strains yielded unique PCR amplification. The sequences of the ITS1-5.8S-ITS2 rDNA region for the thirteen strains were from 527 bp to 631 bp. *Chrysosporium* state of *Ctenomyces serratus* 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 thirteen strains analyzed. The data were also compared with sequences deposited in the NCBI/Genbank for identification purposes and a tree was constructed with the nearest type of strain sequences based on rRNA gene sequences (ITS region) using the Maximum Composite Likelihood Method (Figure 3). The Phylogenetic tree indicates different clusters for each isolate showing their sequence variation. The sequences obtained show greater than 96% similarity to the reference sequences available in NCBI Genbank database.

Chrysosporium indicum (14.00%) was the most prevalent species and this is in agreement with the finding of previous workers [17–20]. Its high percentage of distribution clearly indicates that it is well adapted to warmer condition of India [21]. *Microsporium gypseum* complex was found next to *C. indicum* in distribution (9.33%) and has been reported from various parts of India. We also encountered *Arthroderma* sp. three times in association with the *Microsporium gypseum* complex, which was made up of the two teleomorph states *Arthroderma incurvata* and *A. gypsea*, but *A. fulva* was altogether absent. However, mating experiments were not conducted to confirm its specific identity. It was reported from Indian soils by Dey and Kakoti [22]. It was further isolated from various parts of India [21, 23–27]. *Chrysosporium tropicum* was next to *M. gypseum* comprising of 4.66% in distribution. It is a cosmopolitan species and has been reported from various parts of India [17, 18, 27, 28].

The other species of *Chrysosporium* isolated were *Chrysosporium evolceanui* (2.66%), *Chrysosporium zonatum* (1.33%), and *Chrysosporium* state of *Ctenomyces serratus* (3.33%). *Chrysosporium evolceanui* was reported from Indian soils as *Trichophyton evolceanui* by Randhawa and Sandhu [17]; then Garg [21] placed it in the genus *Chrysosporium* as *C. evolceanui*. It is reported from Indian soils [26, 29, 30]. Various workers have isolated *Chrysosporium zonatum* from Indian soil [31, 32]. *Chrysosporium* state of *Ctenomyces serratus* was recorded in 3.33% of samples and this has been reported from various parts of India [17–19, 21].

Other species of keratinophilic fungi isolated were *Gymnascella dankaliensis* (1.33%), *Gymnascella hyalinospora* (0.66%), *Gymnoascoideus petalosporus* (0.66%), *Auxarthron conjugatum* (2.00%), and *Uncinocarpus queenslandicus* (2.00%). *G. dankailensis* is reported from Indian soils [29, 33]. *Gymnascella hyalinospora* was reported from Indian soils by Singh and Kushwaha [32] and Deshmukh [34]. *Gymnoascoideus petalosporus* was previously recorded from Chilka Lake soil [35], soils from Vedanthangal Water Bird Sanctuary [20], and soils of Orissa [33]. *Auxarthron conjugatum* is reported from Indian soils by Deshmukh and Verekar [20], Jain and Agrawal [36], and Deshmukh and Agrawal [37]. Anamorphic state of *Uncinocarpus queenslandicus* i.e *Chrysosporium queenslandicum* was reported from Indian soil [18, 24, 26, 38, 39].

The other species of dermatophytes recovered were *Trichophyton mentagrophytes* (2.00%) and *T. terrestre* (3.33%). *Trichophyton mentagrophytes* has been reported from Indian soils [26, 34, 40–42] and is known to cause diseases in man and animals [43, 44]. *T. terrestre* has been reported from Indian soils by various workers [26, 45–47].

The prevalence of these fungi in the soils of region of Vidarbha is of importance for their pathogenic potential

FIGURE 3: Phylogenetic tree based on ITS1-5.8S-ITS4 region sequences for isolated 13 keratinophilic fungi with reference strains. Numbers at the respective nodes are percentage of 1000 bootstrap replicates. Bar indicates genetic distance due to sequence variation.

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 [48]. 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 [49]. *Chrysosporium tropicum* was reported from comb lesion in two different breeds of chicken in India [50]. There are reports of disseminated infections due to *C. queenslandicum* in garter snakes [51]. *Gymnascella dankaliensis* was reported from superficial infections in human beings [52] and Iwen et al., [53] isolated *Gymnascella hyalinospora* from invasive pulmonary infection in a patient with

acute myelogenous leukemia. Similarly, Lysková [54] isolated *Chrysosporium queenslandicum*, *C. sulfureum*, *C. tropicum*, *Malbranchea pulchella*, and *Myriodontium keratinophilum* from infections of the skin and nails of patients in the Moravian-Silesian Region (Czech Republic). Thus, these fungi may be regarded as opportunistic pathogens.

There are some reports of occurrence of keratinophilic fungi from various parts of Maharashtra [40, 47, 55, 56], but to best of our knowledge, this is the first comprehensive report on incidence of keratinophilic fungi from Vidarbha region. These findings are in agreement with the findings of previous work on keratinophilic fungi. It appears from this study that a rich variety of keratinophilic fungal flora exists

in Vidarbha region of Maharashtra which may be attributed to the climatic and environmental conditions such as the soil type, vegetation, fauna, and human habitations. Garg et al. [57] emphasized that the climate and perhaps other environmental factors are important in determining the distribution of keratinophilic fungi in ecological habitats.

Our findings confirm the presence of keratinophilic and related dermatophytes in the soils of Vidarbha region. These areas potentially have a high risk for causing cutaneous fungal infections in humans and animals and could be considered as a source of these infections.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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