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Isolation and identification of dermatophytes from soil samples of Jabalpur city

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Abstract

A total of 20 soil samples were collected from ten poultry farms and ten from hair-dumping/garbage sites from different areas of Jabalpur city and were examined for the presence of dermatophytes and related keratinophilic fungi. Twenty (100%) out of 20 soil samples examined were positive for dermatophytic fungi. Results showed that numbers of fungi were more in poultry farms, while the number of species isolated from hair dumping sites were less in number. Twenty three species belonging to various genera were identified from various samples of poultry farms and hair dumping sites.

Keywords: dermatophytes, hair baiting technique, Jabalpur, keratinolytic, keratinophilic

Introduction

Keratinophilic fungi are a specialized group of fungi, which grow on specific keratinous substrate containing complex nitrogenous material. The word keratinophilic means “keratin loving”. These fungi cannot be seen by the naked eye unlike other macro fungi. Keratinophilic fungi which includes dermatophytes are potentially pathogenic to man and animals. During the past few decades, a great interest has developed in medical mycology throughout the world with increasing studies on epidemiology. With few exceptions almost all the keratinophilic fungi grow saprophytically on keratinous substrate. Keratinophilic fungi are present in environment with variable distribution patterns that depends on different factors such as human and animal presence. Keratinophilic fungi are restricted to keratinous substrates like hair, nails and hoof etc. present in soil. The enrichment of soil with keratinous material is most conducive to the growth and occurrence of these fungi (Otcenasek, 1978; Pandhye *et al.* 1967) [4, 5]. The saprophytic growth and survival of dermatophytes largely depends upon the keratinophilic ability, transition from saprophytes to the parasitic life style. Indian habitat being tropical, harbours enormous number of animals and human beings that produce keratin and hence enormous diversity of keratinolytic fungi too. These fungi have been sporadically studied and isolated as compared to other countries (Currah, 1985; Guarro *et al.* 1999; Ulfig, 2003) [1, 2, 8]. The distinction between keratinolytic and keratinophilic fungi is based on keratin usage or destruction. Keratinolytic fungi are a group of microorganisms able to decompose keratin remains in the environment and being potentially pathogenic to human and animals. On the other hand keratinophilic species only use materials associated naturally with keratin or resulting from its destruction. Dermatophytes belong to a large group of keratinophilic fungi and cause human and animal mycoses thus have drawn the attention of medical and veterinary epidemiologists. Thus only fungi that are able to degrade keratin should be considered as keratinophiles or keratinolytic fungi. Two groups the mitosporic fungi Deuteromycetes and Ascomycetes have keratinolytic members that occur commonly in soil as keratin decomposers.

Materials and Methods

Collection of soil samples

Jabalpur is a city found in Madhya Pradesh, India. It is located 23.17 latitude and 79.95 longitude and it is situated at elevation 416 meters above sea level. 20 soil samples were collected from ten poultry farms and ten hair-dumping/garbage sites from different areas of Jabalpur in pre-sterilized plastic bags (10 x 20 cm). Surface soil (depth not exceeding 2 - 3

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original moisture and kept in culture room at a temperature of $28 \pm 2^\circ\text{C}$ till needed. A part of the soil sample was used for baiting and for survival studies of the selected fungi.

Baiting of soil samples

Each soil sample was thoroughly homogenized and a sufficient amount of soil was taken in separate sterilized petri dishes from each sample. Hair baiting technique (Vanbreuseghem, 1952)^[9] was used for isolating the fungi. For that sterilized distilled water was added to provide moisture to the soil. Bits of sterilized human hair and nail were used as baits. The hair and nail were scattered uniformly only on wet soil. Each Petri dish was separately labeled indicating the date, site of collection and type of bait etc. Each Petri dish was incubated at $28 \pm 2^\circ\text{C}$ for 3 to 4 weeks in the culture room. Fungal growth, if any, on the hair and nail bait was observed periodically.

Isolation, purification and identification of fungi

The baited cultures were examined after 3 - 4 weeks for the development of any fungal growth on the hair and nail bait. For fungal examination, a small portion of the fungal growth was picked up with the help of a sterilized needle, mounted on a slide under covered glass containing a drop of sterilized distilled water or any other staining solution, and examined under a microscope for the identification of the fungi. After a preliminary examination of fungal growth on baits, the fungus was subsequently transferred to the slants of Sabouraud's Dextrose Agar (SDA) fortified with chloramphenicol (0.05 mg/ml) and cycloheximide (0.5 mg/ml) to check the bacterial and saprophytic fungal growth, respectively. If mixed growth of fungi occurred, a dilute suspension of the material was transferred on SDA Petri dishes in triplicates. After an incubation of 24 h, the single germinating spores with initial hyphal growth were removed using a sterilized long needle and transferred to fresh slants of SDA medium. In this way, mixed cultures were made pure. Hyphal or spore measurements were recorded using an oculometer in the eyepieces of a

microscope. Whenever essential, photomicrographs were also taken to get a clear picture about the microorganism.

The measurements, shape, arrangements of spores and other structures were also taken. The color, texture, pigmentation on reverse side of colony and colony characters were recorded for fungal identification.

Screening for keratinolysis: The fungi were tested for secondary screening for keratinolysis through keratin invasion test (Salkin *et al.* 1985)^[6].

Determination of keratinolytic potential: The keratinase production was accompanied by keratin degradation and quantitatively determined by weight loss method.

Results

Identification of fungal species

A total of 20 soil samples were collected from ten poultry farms and ten hair-dumping/garbage sites from different areas of Jabalpur. Twenty (100%) out of 20 soil samples examined were positive for keratinophilic/dermatophytic fungi. Fungi isolated from poultry farms and hair dumping sites of various places of Jabalpur city using SDA or PDA media were listed in Table 1. Results showed that numbers of fungi were more in poultry farms, while, the number of species isolated from hair dumping sites were less in number. Twenty three species belonging to various genera were identified from samples of poultry farms and hair dumping sites.

Different species belonging to different genera which includes *Microsporum canis*, *Trichophyton verrucosum*, *Microsporum gypseum*, *Trichophyton tonsurans*, *Candida albicans*, *Chrysosporium* spp., *Trichophyton ajelloi*, *Mucor hiemalis*, *Fusarium solani*, *Cladosporium cladosporoides*, *Aspergillus* spp., *Alternaria alternata*, *Aspergillus flavus*, *Chrysosporium keratinophilum*, *Chrysosporium tropicum*, *Chrysosporium aspartatum*, *Chrysosporium evolceanui*, *Fusarium oxysporum*, *Penicillium* spp., *Fusarium* spp., *Aspergillus niger*, *Cryptococcus* spp., yeasts and moulds.

Table 1: No. of fungi isolated from poultry farms and hair dumping sites of Jabalpur city

S. No.	Sources of Samples	Location	Fungal sp. Isolated	Fungal sp. Identified	Fungal sp. Unidentified
1	Brhad Hauturies	Khamria Road	12	5	7
2	Shubh Poultry	Jamtara	8	4	4
3	Avian Poultry	Shakti Nagar	14	6	8
4	New Patel Poultry	Barghi	6	5	1
5	Sanjay Poultry	Madan Mahal	3	3	0
6	Verma and Son Poultry	Ananad Nagar	8	6	2
7	SB Poultry farm	Tilwara	7	4	3
8	Manoj Poultry	Ranjii	10	4	6
9	National Poultry	Gwahri Ghat	8	6	2
10	Habitat Poultry farm	Ktangi	12	5	7
11	Medical University	Hair Dumping site	17	6	11
12	Opposite civil line police station	Hair Dumping site	10	3	7
13	Sadar Bazar	Hair Dumping site	5	5	0
14	Jabalpur Railway station	Hair Dumping site	7	4	3
15	Jabalpur Bus stand	Hair Dumping site	9	6	3
16	Adhartal	Hair Dumping site	6	5	1
17	Raddi Chowki	Hair Dumping site	4	2	2
18	Ghanta Ghar	Hair Dumping site	5	3	2
19	Nya Mhalla	Hair Dumping site	4	4	0
20	Ghamapur	Hair Dumping site	6	5	1



Fig 1: Medical University

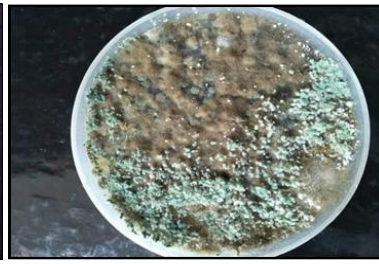


Fig 2: Opposite civil line police station



Fig 3: Sadar Bazar



Fig 4: Jabalpur Railway station



Fig 5: Jabalpur Bus stand



Fig 6: Adhartal

Fig 1-6: Growth of various fungal species on Sabouraud's dextrose agar (SDA) media

Were isolated from poultry farms and hair dumping/garbage sites as given in Figure 1-6. PDA, SDA were the best solid culture media for all the isolates in terms of mycelia growth and sporulation. All the fungal isolates demonstrated keratinolytic activity against hair, feather and nail. The rate of degradation was faster in feather and hair as compared from nail. Sharma (1989) ^[11] reported the dominance of *Trichophyton* species in soil of Jaipur. Rajak *et al.* (1989) ^[12] reported the prevalence of keratinophilic fungi from soil of gelatin factory campus at Jabalpur (Madhya Pradesh, India). They isolated *Microsporium* spp. *Absidia* spp. and *Aspergillus* spp. etc. The soil of Andaman – Islands harbours *Microsporium gypseum*, *Trichophyton terrestre* and five species of *Chrysosporium* (Dixit and Kushwaha, 1990) ^[10]. Kushwaha *et al.* (2001) ^[13] reported keratinophilic fungi in human hair from water sediments of India viz., *Acremonium* sp., *Chrysosporium indicum*, *C. keratinophilum*, *Microsporium gypseum*, *Chrysosporium georgii* and *C. xerophilum*. Ulfing (1994) ^[14] reported the occurrence of keratinophilic fungi viz., *Chrysosporium pannicola*, *Chrysosporium* and anamorph of *Arthroderma curreyi*, *C. keratinophilum*, *Geomyces pannorum*, *Aspergillus flavus*, *Geotrichum candidum*, *Microsporium canis*, *M. cookei*, *Trichophyton ajelloi* and *T. beigeli* from the polluted environment of the Labele district in Gliwice (Poland). Ventkatesan *et al.* (2007) ^[15] reported some dermatophytes viz., *Trichophyton mentagrophytes*, *T. rubrum*, *Epidermatophyton floccosum* and *Microsporium gypseum* on skin and nail scraping from the inhabitants of Chennai, India. (Iqbal *et al.* 2015) ^[16] isolated four fungal species and identified from soil samples collected from

different places of Jabalpur (Madhya Pradesh). *A. niger* was most frequent 60% followed by *A. flavus* (20%), *P. notatum* (10%), *F. oxysporium* (10%).

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