Diversity of Keratin Degrading Fungal Flora in Industrial area of Jaipur and Keratinolytic Potential of Trichophyton Mentagrophytes and Microsporum Canis

Tarun Kumar Kumawat, Vishnu Sharma, Ruchi Seth and Anima Sharma

Department of Biotechnology, JECRC University, Jaipur, Rajasthan, India.

Abstract

Keratinophilic fungi are small but well-defined and important group of fungi that live in soil. Keratinophilic fungi play an important ecological role in decomposing keratins. They produce the keratinase enzyme, which is consisted of disulphide and hydrogen bonds. The soil keratinophilic fungi are responsible for the breakdown of any keratin containing wastes such as hair, nail, fur and feather. The keratinophilic fungi are biggest group of organisms that can utilize keratin as the sole source of carbon and nitrogen. Keratinolytic enzymes from fungal flora may have important uses in biotechnological processes involving keratin-containing wastes from poultry and leather industries. In the present investigation 12 fungal strains were isolated from 58 soil samples. Among them Trichophyton mentagrophytes and Microsporum canis were producing highest quantity of keratinolytic enzyme. Optimum physical condition for the enzyme production were tried to be identified in the present research work. The fungal digestion of animal horn, chicken feathers, finger nails, animal hairs and human hairs suspended in agar-based media by keratinophiles such as Microsporum gypseum, Microsporum canis, Trichophyton mentagrophytes and Trichophyton rubrum has been describe. The distribution and occurrence of keratinophilic fungi from poultry farm’s soil have a role in degradation of keratinous material as an industrial point of view.

Keywords: keratinophilic fungi, keratinase, Trichophyton mentagrophytes and Microsporum canis.
1. Introduction
Sometime in the before time, vertebrates when originated from life in water to life on land, they developed a specialized protein named known as keratin. Keratins are insoluble proteins found in feathers, wool, hooves, scales, hair, nails (hard keratins) and stratum corneum (soft keratins). Due to the strength and stability of keratin a very few organisms are able to break it down and utilize it. In planet several causative fungal species are present. Among them, keratenophills are biggest group of organisms that can utilize keratin as the sole source of carbon and nitrogen. They have two imperative properties as keratinophilic and keratinolytic activity which consumes keratin as their nutrient substrate. Keratinolytic play an important ecological role in decomposing α-keratins, the insoluble fibrous protein. They produce the keratinase enzyme. Among fungi, keratinases are particularly produced by Microsporum, Trychophyton, Doratomyces and microsporum. The aim of this work was to identify the presented keratinophiles in road side soil of Jaipur city and investigation of production level of enzyme with in optimize physical conditions to verify the industrial applications. The present study is intended to differentiate the keratinase activity of Trichophyton mentagrophytes and Microsporum canis isolated on keratin substrates such as human hair, human nail and chicken feather at variable environmental conditions of temperature, pH and metal ions.

2. Materials and Method
2.1 Collection of soil Samples
The selection of study for collecting soil samples was based on contamination of these areas with the higher level of industrial keratin waste. Soil samples were collected in sealed polyethylene bags (10 X 20 cm) using a sterile spatula from different sites of Jaipur city. The soil was air dried overnight for fungal isolation purposes.

2.2 Isolation of keratinophilic fungi from soil
The keratinophilic fungi were isolated using ‘hair baiting technique’ (R. Vanbreuseghem, 1952). The keratinolytic nature of these fungi makes it possible to isolate them from soil by implanting hair, feathers, claws and nails as keratin source. In this technique sterile Petri plates were half filled with soil and short strand of sterilized defatted human hair, nails, chicken feathers were spread over the surface of soil. 10-12 ml. sterile water was added to petri plates for the facilitation of fungal spores to germinate. Now the petri plates were incubated at 20-25°C in dark for 3-4 weeks. After vigorous growth inoculums was placed over the sabouraud’s dextrose agar media.

2.3 Identification of isolated fungi
After the preliminary examination of fungal growth the fungus was subsequently transferred to the slants of sabouraud’s Dextrose Agar Medium (SDA) supplemented with antibiotics for checking the growth of bacteria and saprophytic fungi. With it
subsequently cultures were transferred to SDA plate in replicas. Essential photomicrographs were taken. The shape, arrangement of spores and other structures were studied. The color, texture, pigmentation on reverse surface of the colony and other colony characteristics were also recorded for fungal identification. Fungal spore were examined under microscope using scotch tape mount method.

2.4 Effect of different temperature, pH and metal ions on keratinase enzyme activity
For determination of the effect of temperature (20, 25, 30 35, 40, 45, 50, 55 and 60°C), pH (5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0) and metal ions (Mn$^{2+}$, Mg$^{2+}$, Ca$^{2+}$, Ba$^{2+}$ and Zn$^{2+}$) on keratinase activity. The fungal samples were cultured on Minimal liquid medium (MSM) supplemented with feather meal. As the fungus grew on MSM, it starts consumption of feather meal and release extracellular keratinase, which was estimated for determining the keratinase activity. Keratinolytic activity of culture filtrates was measured spectro-photometrically according to the method given by Takiuchi et al., (1982) with some modifications.

3. Results
The present study emphasize on the distribution and keratinase enzyme activity of keratinophilic fungi. For the isolation of keratinophilic fungi Sabouraud’s dextrose medium was used. Fungal colonies were isolated and identified microscopically. In the present investigation 12 fungal strains were isolated from 58 soil samples. Following keratinophilic species were recorded predominantly: Microsporum canis, Microsporum gypseum, Trichophyton rubrum, Trichophyton mentagrophytes, Epidermophyton floccosum, Chrysosporium tropicum, Chrysosporium keratinophilum, Aspergillus fumigates, Fusarium oxysporum, Fusarium moniliforme, Histoplasma capsulatum and Torula species. (Table-1)

<table>
<thead>
<tr>
<th>Habitats</th>
<th>Chrysosporium tropicum</th>
<th>Chrysosporium keratinophilum</th>
<th>Trichophyton mentagrophytes</th>
<th>Trichophyton rubrum</th>
<th>Microsporum gypseum</th>
<th>Microsporum canis</th>
<th>Aspergillus fumigatus</th>
<th>Fusarium oxysporum</th>
<th>Fusarium moniliforme</th>
<th>Histoplasma capsulatum</th>
<th>Torula species</th>
<th>Epidermophyton floccosum</th>
<th>Positive soil samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
</tr>
</tbody>
</table>

Table 1: Distribution of individual fungal species in different habitats and percentage occurrences.
Out of these 12 species, *Trichophyton mentagrophytes* was isolated from maximum number of soil sample i.e., ten (12.5%) while as *Microsporum canis* was at second position 10.0% in eight soil samples. It was also observed that *Microsporum canis* was not found in soil samples of poultry farms.

### Table 2: Effect of temperature, pH and metal ions concentration on keratinase activity of proteinase of *Trichophyton mentagrophytes* and *Microsporum canis*.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Trichophyton mentagrophytes</th>
<th>Microsporum canis</th>
<th>Trichophyton mentagrophytes</th>
<th>Microsporum canis</th>
<th>Trichophyton mentagrophytes</th>
<th>Microsporum canis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roadside</td>
<td>2</td>
<td>14.28</td>
<td>2</td>
<td>11.1</td>
<td>1</td>
<td>14.8</td>
</tr>
<tr>
<td>Poultry farm</td>
<td>3</td>
<td>21.42</td>
<td>2</td>
<td>16.6</td>
<td>2</td>
<td>40.0</td>
</tr>
<tr>
<td>Household dust</td>
<td>2</td>
<td>14.28</td>
<td>2</td>
<td>16.6</td>
<td>4</td>
<td>22.2</td>
</tr>
<tr>
<td>Animal habitat</td>
<td>4</td>
<td>38.56</td>
<td>4</td>
<td>33.3</td>
<td>2</td>
<td>25.0</td>
</tr>
<tr>
<td>Pigeon habitat</td>
<td>3</td>
<td>21.42</td>
<td>2</td>
<td>16.6</td>
<td>6</td>
<td>22.2</td>
</tr>
<tr>
<td>Industry</td>
<td>-</td>
<td>18.15</td>
<td>1</td>
<td>15.2</td>
<td>10</td>
<td>11.2</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>17.15</td>
<td>1</td>
<td>15.2</td>
<td>10</td>
<td>9.0</td>
</tr>
</tbody>
</table>

| | | | |
| | | | |
| | | | |

Out of these 12 species, *Trichophyton mentagrophytes* was isolated from maximum number of soil sample i.e., ten (12.5%) while as *Microsporum canis* was at second position 10.0% in eight soil samples. It was also observed that *Microsporum canis* was not found in soil samples of poultry farms.
In the present study *Trichophyton mentagrophytes* showed high enzyme activity (2.57 unit/ml) at 35°C in temperature, (3.17 unit/ml) at 7.5 in pH and (3.89 unit/ml) at Mg\(^{2+}\) in metal ion concentration. While *Microsporum canis* showed high enzyme activity (2.11 unit/ml) at 35°C in temperature, (2.14 unit/ml) at 7.5 in pH and (3.16 unit/ml) at Mg\(^{2+}\) in metal ion concentration.

### 4. Discussion

Keratinophiles from nature require various sampling methods and isolation techniques. In nature soil represent an incessant reservoir of keratinophytes for their saprophytic life. Sharma et al., 2011 studied the differences in keratinase enzyme activity in *T. mentagrophytes*, *T. rubrum*, *Microsporum canis* and *M. gypseum* isolated on keratin substrates. They also elucidated to various environmental condition such as pH and metal ions. Similarly Farzana 2007, was surveyed the prevalence of *Tricophyton* and *Microsporum* at Rajshahi, Bangladesh. pH is the most important factor, which markedly influence enzyme activity. Extremely high and low pH values generally complete loss of activity for most of enzymes. Gupta et al., 2010 characterized the production of protease (keratinase) at various physical conditions as pH and metal ions (Hg\(^{2+}\), Ag\(^{2+}\), and Zn\(^{2+}\)) for *Microsporum canis*.

### 5. Conclusion

The soil samples are rich in diversity of keratinophilic fungi with high abundance of *Trichophyton mentagrophytes* and *Microsporum canis*. Both are geophilic dermatophytic fungi which produced keratinase enzyme that can be used in biotechnological processes.
6. Acknowledgement
The authors are indebted to Head, Department of Botany, University of Rajasthan, Jaipur. Grateful acknowledgement is also due to JECRC University, Jaipur for giving us support during research work.

References