

Comparative Study of Keratinolytic Activities of Dermatophytes in Various Keratin Substrates

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Abstract

Objective: Keratinolytic activities of dermatophytes have been a subject of interest to understand the pathogenicity of infection. In this study, we intend to elucidate the keratinase activity profile among the *Trichophyton* (T), *Microsporium* (M) and *Epidermophyton* (E).

Methods: 343 clinical isolates of dermatophytes viz; *T. mentagrophytes* (228), *T. rubrum* (58), *T. schoeleinii* (36) *M. audouinii* (14) and *E. floccosum* (7) grown on Sabouraud Dextrose broth were inoculated on mineral medium consist of human hair, horse hair, cow hair, chicken feather and fowl scales individually. The keratinolytic activity was measured spectrophotometrically at 660 nm following Folin-Ciocalteu method.

Results: The keratinolytic activities of *T. mentagrophytes*, *T. rubrum*, *T. schoeleinii* and *M. audouinii* of Mean \pm SEM viz; 4.89 ± 5.77 , 4.51 ± 2.51 , 4.57 ± 8.81 and 4.67 ± 8.81 , respectively were significantly increased in feather except, *E. floccosum* with an expression of 1.50 ± 2.08 which was significantly low when compared with other keratin $P < 0.05$. There was a significant decrease in keratinolytic activities of all the dermatophytes in fowl scales $P < 0.05$.

Conclusion: We concluded that the pathogenicity potential of dermatophytes depends on its keratinolytic activities and the structure of the keratin material.

Keywords: Keratinolytic; Dermatophytes; Keratin ; *Trichophyton*; *Microsporium*; *Epidermophyton*

Introduction

Dermatophytes are a group of closely related fungi that have the capacity of invading the keratinized tissue (skin, hair and nails) of human and other animals to produce infections known as dermatophytoses, which are commonly referred to as ringworm [1].

Dermatophytes can digest keratin and other proteinaceous substrates present in Skin and its appendages, such as nail, hair, and feather, and use it as its sole source of carbon and nitrogen. Proteolytic and keratinolytic activities of dermatophytes have been a subject of interest for several years to understand the pathogenicity of infection [2].

These dermatophytes are also called keratinophilic fungi because of their high affinity for keratin. Keratin is a refractory protein polymer only produced by man and animal, it is the main constituent of epidermal skin, hair, feather, reptilian scales, quills, horns, hooves and nails. When these materials are shed into the soil and other potentially moist substrata such as disused nest, they are principally degraded by keratinophilic fungi [3].

Dermatophytes of different anatomical groups have evolved, owing to their differences in site and geographical location specificity. However, a detailed comparative study of the selective preference of the different native keratin substrates among the anatomical groups of dermatophytes and its possible co-relation to pathogenicity is lacking. Therefore, in the current study, we intended to elucidate the differences in the keratinolytic activities of *T. mentagrophytes*, *T. rubrum*, *T. schoeleinii*, *M. audouinii* and *E. floccosum* on keratin substrates such as human hair, horse hair, cow hair, chicken feather and fowl scales.

Materials and Methods

Three hundred and forty-three clinical isolates of dermatophytes

viz; *T. mentagrophytes* (228), *T. rubrum* (58), *T. schoeleinii* (36) *M. audouinii* (14) and *E. floccosum* (7) grown on Sabouraud Dextrose broth were used in the current study. Twenty micro liters of the fungal spore suspension of the dermatophytes viz. *T. mentagrophytes* *T. rubrum*, *T. schoeleinii* were prepared in phosphate buffer solution (PBS) pH 7.8, adjusted to 0.5 Macfarlan' standard were used as inoculums for the present study. Conical flasks containing 1% of different native keratin substrates viz. Human hair, horse hair, cow hair, chicken feather and fowl scales individually for each isolate of each species.

The above substrates viz. Human hair, horse hair, cow hair, chicken feather and fowl scales were washed with ethanol, dried, and hammer milled individually prior to addition to the medium. Overnight broth culture of dermatophytes was filtered into a sterile test tube. From the above test tube, 3.0 ml of culture filtrate was dispensed into another sterile test tube, 3.0 ml of Phosphate buffer solution (PBS) was added and 3.0 ml of 1% caseins in 25 ml test tube, which was put in water bath at 35°C for 1 hr. After the reaction, 5 ml of 20% Trichloroacetic acid (TCA) was added to stop the reaction. The solution was then filtered by Whatman filter paper 540 (Ashless). To 1 ml of filtrate (enzyme substrate mixture), 2 ml of 20% sodium carbonate (Na_2CO_3) was added. 1 ml of folin-ciocalteu reagent was also added and the content was mixed

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immediately. After 30 mins, 6 ml of distilled water was added and mixed thoroughly. The mixture was measured spectrophotometrically at 650 nm. The amount of amino acid released was calculated from a standard curve plotted from known concentration of tyrosine per/ml and this is defined as one unit of the enzyme, from the mixture of keratin and crude enzyme, 1 ml filtrate was transferred into a test tube and 2.0 ml of sodium carbonate was added and this was mixed thoroughly and 0.5 ml of folin was added.

This was incubated for 30 mins at 28°C. The OD was read spectrophotometrically at 660 nm. The keratin filtrate without the crude enzyme was used as a control/blank. Different keratin such as human hair, horse hair, cow hair feather, fowl scales were used as a keratin source and keratinolytic activities was determined in order to know which of the substrates will induce the highest keratinolytic activities [4].

Results

Comparison of keratinolytic activities of dermatophytes in various keratin substrates showed that the keratinolytic activities of *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Trichophyton schoeleinii*, *Microsporium audouinii* and *Epidermophyton floccosum* were significantly higher in feather when compared with other keratin P<0.05. Also it was statistically shown that there was a significant decrease in keratinolytic activities of all the dermatophytes in fowl scales (Tables 1-3).

Comparison of keratinolytic activities of dermatophytes in human hair showed that there was a significant increase in the keratinolytic activities of the *Trichophyton mentagrophytes* in human hair than other dermatophytes. F-value 9.183, P<0.05 (Table 4).

Comparison of keratinolytic activities of dermatophytes horse hair showed that there was no significant difference in keratinolytic activities of the dermatophytes in horse hair. F-Value 0.546, P>0.05 (Table 4).

Comparison of keratinolytic activities of dermatophytes in cow hair showed that there is a significant increase keratinolytic activity of *Trichophyton rubrum* in cow hair than other dermatophytes. F-value 3.3241, P<0.05 (Table 4).

Comparison of keratinolytic activities of dermatophytes in feather showed that there is a significant increase in keratinolytic activities of the *Trichophyton mentagrophytes* in feather than any other dermatophytes. F-value 9.873, P<0.05 (Table 5).

Comparison of keratinolytic activities of dermatophytes in fowl skin showed that there was no significant difference in the keratinolytic activities of all the dermatophytes tested in fowl skin. F-Value 0.1670, P>0.05 (Table 5).

| Natural keratin substrate | N | <i>T. mentagrophyte</i> keratinolytic activities (unit /ml) Mean ± SEM | N | <i>T. rubrum</i> keratinolytic activities (unit /ml) Mean ± SEM |
|---------------------------|-----|--|----|---|
| Human hair | 228 | 1.96 ± 5.77 | 56 | 1.83 ± 1.45 |
| Horse hair | 228 | 3.55 ± 8.82 | 56 | 3.55 ± 1.45 |
| Cattle hair | 228 | 3.55 ± 1.20 | 56 | 3.61 ± 1.76 |
| Feather | 228 | 4.89 ± 5.77 | 56 | 4.51 ± 2.51 |
| Fowl skin | 228 | 0.14 ± 8.81 | 56 | 0.16 ± 1.20 |
| F value | | 45132.27 | | 45132.27 |
| P – value | | <0.05 | | <0.05 |

Table 1: Comparison of Keratinolytic activities of *Trichophyton mentagrophyte* and *Trichophyton rubrum* among various keratin substrates

| Natural keratin substrate | N | <i>T. schoeleinii</i> Keratinolytic activities (unit /MC) Mean ± SEM | N | <i>M. audouinii</i> keratinolytic activities (unit /ml) Mean ± SEM |
|---------------------------|----|--|----|--|
| Human hair | 36 | 1.77 ± 5.77 | 14 | 1.85 ± 1.52 |
| Horse hair | 36 | 3.54 ± 3.18 | 14 | 3.56 ± 3.33 |
| Cow hair | 36 | 3.56 ± 1.15 | 14 | 3.57 ± 8.81 |
| Feather | 36 | 4.57 ± 8.82 | 14 | 4.68 ± 8.81 |
| Fowl skin | 36 | 0.17 ± 1.20 | 14 | 0.17 ± 1.20 |
| F value | | 10888.71 | | 28751.87 |
| P–value | | <0.05 | | <0.05 |

Table 2: Comparison of Keratinolytic activities of *Trichophyton schoeleinii* and *Microsporium audouinii* among various keratin substrates

| Natural Keratin substrate | N | <i>Epidermophyton floccosum</i> keratinolytic activities (unit /ml) Mean ± SEM |
|---------------------------|---|--|
| Human hair | 7 | 1.67 ± 1.15 |
| Horse hair | 7 | 3.57 ± 1.20 |
| Cow hair | 7 | 3.38 ± 2.31 |
| Feather | 7 | 4.50 ± 2.08 |
| Fowl skin | 7 | 0.16 ± 8.81 |
| F value | | 11287.26 |
| P–value | | <0.05 |

Table 3: Comparison of Keratinolytic activities of *Epidermophyton floccosum* among various keratin substrates

| Dermatophytes | N | Human hair keratinolytic (unit /ml) Mean ± SEM | Horse hair keratinolytic (unit /ml) Mean ± SEM | Cow hair keratinolytic activities (unit / ml) Mean ± SEM |
|-----------------------------------|-----|--|--|--|
| <i>Trichophyton mentagrophyte</i> | 228 | 1.96 ± 5.77 | 3.55 ± 8.81 | 3.55 ± 1.20 |
| <i>Trichophyton rubrum</i> | 58 | 1.86 ± 1.45 | 3.55 ± 1.45 | 3.61 ± 1.76 |
| <i>Trichophyton schoeleinii</i> | 36 | 1.77 ± 5.77 | 3.54 ± 3.18 | 3.56 ± 1.15 |
| <i>Microsporium audouinii</i> | 14 | 1.85 ± 1.528 | 3.56 ± 3.33 | 3.57 ± 8.81 |
| <i>Epidermophyton floccosum</i> | 7 | 1.67 ± 1.155 | 3.57 ± 1.20 | 3.38 ± 2.3 |
| F value | | 9.183 | .546 | 3.3241 |
| P–value | | <0.05 | >0.05 | <0.05 |

Table 4: Comparison of keratinolytic activities of dermatophytes in human hair, horse hair and cow hair

| Dermatophytes | N | Feather Keratinolytic activities (unit /ml) Mean ± SEM | Fowl skin Keratinolytic activities (unit/ml) Mean ± SEM |
|-----------------------------------|-----|--|---|
| <i>Trichophyton mentagrophyte</i> | 228 | 4.89 ± 5.77 | 0.14 ± 8.81 |
| <i>Trichophyton rubrum</i> | 58 | 4.51 ± 2.51 | 0.16 ± 1.20 |
| <i>Trichophyton schoeleinii</i> | 36 | 4.57 ± 8.81 | 0.17 ± 1.20 |
| <i>Microsporium audouinii</i> | 14 | 4.67 ± 8.81 | 0.17 ± 1.20 |
| <i>Epidermophyton floccosum</i> | 7 | 1.50 ± 2.08 | 0.16 ± 8.81 |
| F value | | 9.873 | 0.1670 |
| P–value | | <0.05 | >0.05 |

Table 5: Comparison of keratinolytic activities of dermatophytes in feather and fowl skin

Discussion

Among the five keratinous viz; Human hair, horse hair, cow hair, chicken feather and fowl scales, Feather was the most preferred keratin source for isolates of *T. mentagrophytes*, *T. rubrum*, *T. schoeleinii* and *Microsporium audouinii*, as all these organisms showed maximum keratinolytic activities in feather of Mean ± SEM viz; 4.89 ± 5.77, 4.51 ± 2.51, 4.57 ± 8.81 and 4.67 ± 8.81, respectively, except *Epidermophyton floccosum* with an expression of 1.50 ± 2.08 which is significantly low P<0.05 Meanwhile, there was no significant difference in keratinolytic

activities of all the dermatophytes in horse hair and fowl scale. This study corroborates the findings of Apocada and Mckerrow [5] in Israel. The present study indicates that animal and birds are equally susceptible to dermatophytes infections. Though the keratinolytic activities of all the dermatophytes tested were significantly low in fowl scales, and this may be due to the constituency of the fowl scale keratin belonging to the hard keratin that are not easily broken by the dermatophytes.

Among these five dermatophytes, it was observed that *Trichophyton mentagrophytes* has significant increased keratinolytic activities in human hair and feather than other dermatophytes. This might be as a result of the presence of the perforating organ in *T. mentagrophytes*, which facilitate the mechanical destruction of keratin and allows the growth of mycelia faster [6]. *Trichophyton rubrum* showed its preference for cow hair among other dermatophytes, the keratinolytic activity was however higher than other dermatophytes in cow hair, which does not correlate with previous finding of Venkantesan et al. [2].

Conclusion

It is concluded that the pathogenicity potential of dermatophytes

depends on its keratinolytic activities and the structure of the keratin material infected.

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