ABSTRACT

Paula [2] demonstrated that all the strains of generally considered to be potential pathogenic factors. Coutinho and a keratinolytic protease of Protease is an important virulence factor of candidiasis in humans and one of the virulence factors possessed by many micro-organisms. M. dermatitis vary quantitatively and there may be possibility of involvement of protease in the manifestation of clinical entities. Human stratum corneum protease activity. Protease released by their study isolated from dogs with otitis and dermatitis showed significance because they may be involved in the pathogenesis of histopathological changes associated with M. dermatitis. The pathogenic role of proteases of Malassezia spp. in various diseases is therefore a continued topic of interest in human and veterinary medical literature.

INTRODUCTION

Lipid-dependent Malassezia species have been reported to elaborate a range of enzymes. These enzymes have been the subject of recent interest because they may be involved in the pathogenesis of clinical disease. Chen et al. [1] established that the culture supernatants of M. sp. used in this study also contained proteases. Enzyme secretion is one of the virulence factors possessed by many micro-organisms. Protease is an important virulence factor of candidiasis in humans and a keratinolytic protease of Candida albicans has been shown to digest human stratum corneum in vitro. Enzymes produced by Malassezia are generally considered to be potential pathogenic factors. Coutinho and Paula [2] demonstrated that all the strains of M. pachydermatis used in their study isolated from dogs with otitis and dermatitis showed protease activity. Protease released by Malassezia was proposed as the mediator of itch at free nerve endings in the skin and a contributor to the prominent pruritus seen in affected dogs.

However, little work has been undertaken to elucidate at the role of proteases in the pathogenesis of histopathological changes associated with M. dermatitis. The pathogenic role of proteases of Malassezia spp. in various diseases is therefore a continued topic of interest in human and veterinary medical literature.

MATERIALS AND METHODS

Isolation of M. furfur

12 cultures were isolated from patients showing clinical conditions of pityriasis versicolor (4), dandruff (4) and seborrheic dermatitis (4). All isolates were identified as M. furfur by their lipid dependent growth. The isolates were found to be positive for urease production and negative for nitrate reduction.

Enzyme study

Proteolytic enzyme assay on the 12 isolates of M. furfur was carried out with slight modification of the method described by Tsujibo et al. [3]. Lipid supplement was provided for M. furfur in the assay medium.

In brief, 0.5 ml of culture filtrate was incubated with 1 ml of 1 % vitamin free casein in Tris buffer (pH 7.5) at 37 °C for 20 minutes. After incubation, enzyme activity was arrested and the protein was precipitated by 5 ml of 20 % trichloroacetic acid (TCA) and was filtered through Whatman No.1 filter paper. The tyrosine in the filtrate was read spectrophotometrically at 280 nm. Similarly, a control was run in an identical manner with the culture filtrate being precipitated by 5 ml of 20 % trichloroacetic acid (TCA) and was precipitated. The optical density value for the enzyme activity was calculated using standard procedure. One unit of proteolytic enzyme activity was the amount of the enzyme, which liberated one µmol of tyrosine/ml/minute under assay condition.

The enzyme activity for each of the test organisms at different intervals (days) of growth was represented in a graph by plotting the protease activity in the ‘Y’ axis and the time interval in ‘X’ axis. The enzyme activity was also compared with the severity of the lesion produced by the clinical isolates.

The details regarding nature of infection, and the severity of lesion etc were recorded for the correlation of the enzyme profile of the organism with the severity of infection.

RESULTS

Category of Clinical infection

Enzyme activity of M. furfur

The enzyme activity was maximum for the M. furfur isolates from the cases of seborrheic dermatitis followed by the isolates from the clinical cases of pityriasis versicolor. However peak enzyme activity in all the isolates from different clinical conditions were recorded on 16 – 20 days.

Original Article

IS MODERATION OF PROTEASE PRODUCTION BY MALASSEZIA FURFUR AN ESSENTIAL ASPECT OF ITS PATHOGENESIS AND VARIED CLINICAL MANIFESTATION

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Multiple Range Tests (Tukey-HSD test) was applied to compare protease activity of the different isolates of M. furfur. The M. furfur isolates from different clinical isolates recorded statistically significant difference in the levels of their enzyme activity (Table 1a and 1b, Fig 1a, 1b).

DISCUSSION

Protease in M. furfur and pathogenesis

Enzyme secretion is one of the virulence factors possessed by many micro-organisms. Protease is an important virulence factor of candidiasis in humans and a keratinolytic protease of Candida albicans has been shown to digest human stratum corneum in vitro. Enzymes produced by Malassezia are generally considered to be potential pathogenic factors. Coutinho and Paula [2] experimentally proved that all the strains of Malassezia pachydermatis isolates from dogs with otitis and dermatitis exhibited protease activity. Protease released by Malassezia was proposed as the mediator of itch at free nerve endings in the skin and a contributor to the prominent pruritus seen in affected dogs. Seborrheic dermatitis is characterized by inflammation and desquamation in areas that are rich in sebaceous glands such as the scalp, face and upper trunk, whereas dandruff is a noninflammatory scaling condition of the scalp [4].

It is now generally considered that the latter is the mildest form or a variant of seborrheic dermatitis. The importance of Malassezia organisms in these two conditions has been supported by studies demonstrating parallel decreases in the number of organisms and the severity of the diseases. Malassezia organisms produce lipases, which alter sebum production and produce free fatty acids on the skin surface [5]. M. pachydermatis strains are known to produce proteases that are linked to its parasitic mode of life. However, little work has been undertaken looking at the role of proteases in the pathogenesis of histopathological changes associated with M. dermatitis [1].

The protease activity of the isolates of M. furfur from different clinical conditions such as pityriasis versicolor, dandruff and seborrheic dermatitis showed varied activity in the present study. The protease production is mild from isolates of pityriasis versicolor, high in dandruff and very high in seborrheic dermatitis.

It is interesting to note that the low protease activity of M. furfur isolates corresponds to chronicity of pityriasis infection, which is in similar line to that of Trichophyton rubrum isolates from chronic cases of dermatophytosis. We have established in our earlier studies [3,6] a similar correlation between chronicity and low protease profile of T. rubrum isolates. In the present study, the protease activity is high in isolates of seborrheic dermatitis, which again corresponds to the high level of inflammation in the patients. The role of protease in pathogenesis or severity of infection caused by M. furfur is not clearly known, however the present findings throw light on the possible role. But in the earlier study conducted by Chen et al. [1] the culture extracts of Malassezia sp. with and without proteases failed to stimulate canine keratinocytes in vitro.

However, the present study suggests that the possible role of proteases in eliciting an immune response in the in vivo condition may not be ruled out. Probably the combined activity of lipases and proteases could be responsible for the clinical manifestations/conditions caused by M. furfur.

**Table 1a: Comparative enzyme activity in M. furfur isolates from different Clinical conditions.**

<table>
<thead>
<tr>
<th>Clinical categories</th>
<th>No. of isolates</th>
<th>Chronic cases</th>
<th>Non-chronic cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pityriasis versicolor</td>
<td>4</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Dandruff</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Seborrheic dermatitis</td>
<td>4</td>
<td>-</td>
<td>4</td>
</tr>
</tbody>
</table>

**Figure (1a) Comparative enzyme activity in M. furfur isolates from different clinical conditions at different days**

**Figure (1b) Comparative enzyme activity in M. furfur isolates from different Clinical conditions at different days**
Table 1b: Comparative enzyme activity in *M. furfur* isolates from different Clinical conditions at different days

<table>
<thead>
<tr>
<th>Days</th>
<th>Enzyme Activity (units)</th>
<th>SD</th>
<th>PV</th>
<th>D</th>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>10</td>
<td>66.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.09</td>
<td>19.50&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>12</td>
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<td>12.91</td>
<td>34.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.84</td>
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<tr>
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<td>16</td>
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<td>22.17</td>
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<tr>
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</tr>
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</table>

CONCLUSION

Protease elaborated by isolates of *M. furfur* from different clinical conditions such as pityriasis versicolor, dandruff and seborrhoeic dermatitis vary quantitatively and there may be possibility (?) of involvement of protease in the manifestation of clinical entities.

REFERENCES