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Original Article

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

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ABSTRACT

Objective: *Malassezia furfur* has been implicated in other skin diseases such as dandruff (pityriasis capitis) and seborrhoeic dermatitis (an inflammatory condition of the scalp areas rich in sebaceous glands.) *M.furfur* is a commensal flora of the human skin and the conditions that influence the change commensal to pathogen state are not fully understood. To understand the differences in the protease activity and its possible role in the pathogenesis of *M. furfur* isolates from different clinical conditions.

Methods: Proteolytic enzyme assay on the 12 isolates of *M. furfur* was carried out spectrophotometrically with slight modification from the method described by Tsujibo *et al.*

Results: The enzyme activity was maximum for *M. furfur* isolates from the cases of seborrhoeic dermatitis followed by the isolates from the clinical cases of dandruff. The minimal activity was recorded from the isolates from the clinical cases of pityriasis versicolor.

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.

Keywords: Protease, *Malassezia furfur,* Pityriasis versicolor, Dandruff and seborrhoeic dermatitis

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 seborrhoeic 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 45 °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 added after precipitation with TCA. The optical density value for the tyrosine in the test was compared with the standard tyrosine graph and the enzyme activity was calculated using standard procedure. One unit of proteolytic enzyme activity was the amount of the enzyme, which liberated one µ mol of tyrosine/ml/minute under assay condition.

The enzyme activity for each of the test organisms at different time 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 seborrhoeic dermatitis followed by the isolates from the clinical cases of dandruff. The minimal activity was recorded from 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.

Statistical analysis

Multiple Range Tests (Tukev- 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 seborrhoeic 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 seborrhoeic 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 seborrhoeic dermatitis.

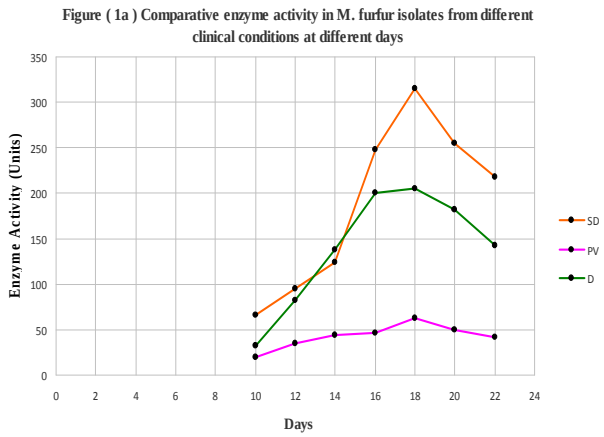
It is interesting to note that the low protease activity of *M. furfur* isolates corresponds to chronicity of pityriasis infection, which is in similar line to that of *Trichophyton rubrum* isolates from chronic cases of dermatophytosis. We have established in our earlier studies [3,6] a similar co-relation between chronicity and low protease profile of *T. rubrum* isolates. In the present study, the protease activity is high in isolates of seborrhoeic 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 throws 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.*

Clinical categories	No. of isolates	Chronic cases	Non- chronic cases
Pityriasis versicolor	4	4	-
Dandruff	4	2	2
Seborrhoeic dermatitis	4	-	4

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

Clinical Conditions	Enzyme Activity (units) Mean	SD
SD	188.57[c]	91.51
PV	42.61[a]	15.46
D	140.36[b]	62.60
F-value	37.075	
P-value	0.000**	



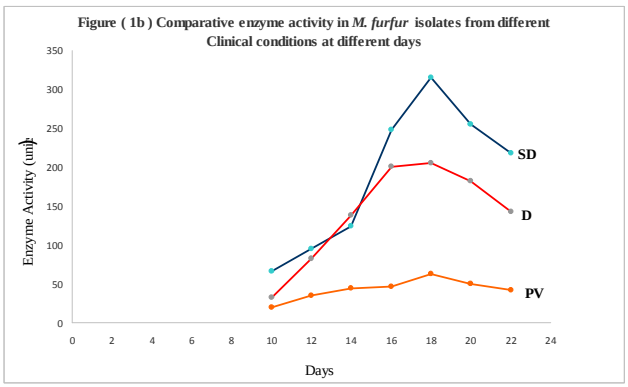


Table 1b: Comparative enzyme activity in *M. furfur* isolates from different Clinical conditions at different days

Days	Enzyme Activity (units)					
	SD		PV		D	
	Mean	SD	Mean	SD	Mean	SD
10	66.25[a]	11.09	19.50[a]	4.20	32.50[a]	7.45
12	95.00[a]	12.91	34.75[ab]	11.84	82.50[b]	13.23
14	123.75[a]	13.77	44.25[bc]	7.59	137.50[c]	22.17
16	247.50[b]	22.17	46.00[bc]	9.52	200.00[e]	18.26
18	315.00[c]	12.91	62.50[c]	3.42	205.00[e]	17.32
20	255.00[bc]	41.23	49.75[bc]	13.87	182.50[de]	17.08
22	217.50[b]	48.56	41.50[abc]	15.15	142.50[cd]	30.96
F-value	47.266		6.673		44.125	
P-value	0.000**		0.000**		0.000**	

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 .

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