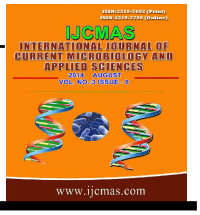
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 9 (2014) pp. 1023-1027**
http://www.ijcmas.com



Original Research Article

Comparative physicochemical properties of partially purified Hemagglutinins from an anthropophilic dermatophyte (*Trichophyton rubrum)* and a zoophilic dermatophyte (*Trichophyton mentagrophytes)*

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Keywords

Trichophyton rubrum, Trichophyton mentagrophytes partially purified Hemagglutinins

A B S T R A C T

Little information are available on the physiological roles of fungal hemagglutinins, though, it seems that fungal hemagglutinins play a role in cell wall biosynthesis, mycelial differentiation. concluded that both the intra and extracellular hemagglutinins from *T. rubrum and T mentagrophytes* are thermally stable and favour alkaline pH for their activities which were independent of metal ions or divalent cations as most of the fungal agglutinins from this study it can be revealed that the knowledge about the physicochemical properties of both the fungal hemagglutinins can be deployed in medical and veterinary mycology to find out their specific roles as infective agent towards human as well as animal host.

Introduction

A large number of fungal species are reported to contain hemagglutinins but only a few of them have been characterized physicochemically. Little information are available on the physiological roles of fungal hemagglutinins , though, it seems that fungal hemagglutinins play a role in cell wall biosynthesis, mycelial differentiation, often involved in adhesion and recognition phenomenon as well as in the interaction of fungi to animals also. (Ishikawa, 1983, 1989; Crandall,1968, Latge 1988).

Some studies regarding preliminary physicochemical properties have been made on some dermatophytes like *Trichophyton rubrum, Trichophyton violaceum, Microsporum gypseum, Chrysosporium keratinophilum* and *Anixiopsis stercoraria* (Rafai, *et.al.*, 1977, 1985; Chabasse *et. al.*, 1986)

The present study deals with the comparison of physicochemical properties of two hemagglutinins , isolated and partially purified from one anthropophilic

dermatophyte, *Trichophyton rubrum* and one from zoophilic dermatophyte *Trichophyton mentagrophytes*. (Mitra and Ghoshal, 2014).

Materials and Methods

The strains of *Trichophyton rubrum* (MTCC-296) and *Trichophyton mentagrophytes* were obtained from the Institute of Microbial Technology, Chandigarh, India and from the Department of Mycology, School of Tropical Medicine, Calcutta, respectively.

The organisms were maintained on Sabouraud's dextrose agar slopes (Sabouraud, 1911). The culture was maintained at $30^0 \pm 0.5^0$C for three weeks. *Trichophyton rubrum* shows heavy growth with creamy white spores accumulation on the surface and dark red pigmentation.

No significant pigmentation appeared in case of *Trichophyton mentagrophytes* below the growth layer. The cultures were preserved at 4^0C. They were transferred to the fresh media at two months intervals.

To obtain uniform inoculum, growth on Sabouraud's agar slope (15 days old) was harvested with sterile distilled water, washed twice and finally suspended in 5ml of sterile water. 0.2ml of the suspension was used as inoculum.

For this study the comparative physicochemical properties of both the haemagglutinines, following parameters were studied. Effect of temperature and pH, Effect of trypsin, Effect of chemicals, Effect of divalent cations, Effect of EDTA.

The effect of temperature and pH, the extracts (1ml each) were incubated separately in a water bath from 10-100^0C for 30 min were studied. Aliquotes (50µl) were withdrawn at regular interval of time with the rise of temperature. After cooling, hemagglutination activity was evaluated against normal human erythrocytes. All extracts were also tested for hemagglutination activity using buffer of various pH (Citrate-phosphate buffer, pH 4-6; PBS-pH 6.8, Tris-HCl, pH 7-10)

The effect of trypsin, hemagglutinins of the two species were treated with 1% trypsin solution, pH 7.3 for 30 min at 37^0C following which the hemagglutination assays were made.

To study the effect of chemicals hemagglutinins of both species were incubated with 100mM of sodium periodate, Sodium citrate, 1% SDS, 1% deoxycholate and 8M urea at room temperature for 30 min and then the hemagglutination activity of the chemically treated materials was tested with normal human erythrocytes.

To study the effect of (divalent cations) calcium in the hemagglutination, samples were examined with Tris buffer, pH 8, containing 10 mM-100 mM $CaCl_2$. Controls without $CaCl_2$ were also tested. Hemagglutination titers were determined against normal human erythrocytes.

To study the effect of EDTA, hemagglutinins of the two fungi were dialyzed extensively against Tris buffer, pH 8 containing 50mM EDTA, re-dialyzed against the same buffer and then the hemagglutination assay was performed with normal human erythrocytes. Controls were set up without EDTA.

Results and Discussion

Results showed that though hemagglutinins of the two species were thermally stable upto 100°C, they were found to be active in

lower temperature (Table-1). Similarly, they were more active in alkaline pH (Table-2) and their activities decreased at pH below 6 and above 10. Both intra and extracellular hemagglutinins of two species were apparently stable to heat. This is possibly due to high sugar content which could protect the protein moiety of the hemagglutinins against heat as has been demonstrated in other dermatophyte agglutinins (Bouchara *et.al.*, 1987).

Trypsin (1%) slightly affected the hemagglutination. Pretreatment of hemagglutinins with 1% trypsin slightly changed their hemagglutination activities indicating that a small portion of the hemagglutinins is protein in nature.

The activities of the hemagglutinins were moderately reduced when treated with Sodium per-iodate and 1% SDS. Remarkable changes occurred by treatment with urea and 1% deoxycholate. 2-marcaptoethanol and sodium citrate did not reduce the activities (Table-3). Pretreatment with periodate moderately affected the hemagglutination activities indicating that carbohydrate parts are responsible for their activities. Drastic reduction of the activities of the agglutinins by detergent presumably due to the breakdown of tertiary structures of the hemagglutinins. Treatment with 2 Markaptoethanol did not affect the hemagglutination properties suggesting that the disulphide bonds (Cysteine) if present, probably did not play any role in the hemagglutination activity.

Hemagglutinins of both species were found to be independent of divalent cation (Ca^{+2}) for their activities; slight decrease in their activities was observed at higher concentrations (Table-4). Treatment with EDTA did not hamper their hemagglutination activity.

Thus, it can be concluded that both the intra and extracellular hemagglutinins from *T. rubrum* and T *mentagrophytes* are thermally stable and favour alkaline pH for their activities which were independent of metal ions or divalent cations as most of the fungal agglutinins from this study it can be revealed that the knowledge about the physicochemical properties of both the fungal hemagglutinins can be deployed in medical and veterinary mycology to find out their specific roles as infective agent towards human as well as animal host.

Table.1 Effect of temperature on the intra and extracellular hemagglutinins from *Trichophyton rubrum* and *Trichophyton mentagrophytes*

Temperature (^{0}C)	Titer[a]			
	Trichophyton rubrum		*Trichophyton mentagrophytes*	
	Intra cellular	Extra cellular	Intra cellular	Extra cellular
10	16	16	16	16
20	16	16	16	16
40	8	8	8	8
60	8	8	8	8
80	4	4	4	4
100	2	2	2	2

Titre [a]: Hemagglutination titer was determined with untreated human erythrocytes.

Table.2 Effect of pH on the activity of intra and extracellular hemagglutinins from *Trichophyton rubrum* and *Trichophyton mentagrophytes*

pH	Titer[a]			
	Trichophyton rubrum		*Trichophyton mentagrophytes*	
	Intra cellular	Extra cellular	Intra cellular	Extra cellular
4	0	0	0	0
5	2	2	2	2
6	4	4	4	4
7	8	8	8	8
8	16	16	16	16
9	16	16	16	16
10	8	8	8	8

Titre [a]: Hemagglutination was determined with untreated human erythrocytes.

Table.3 Effect of chemical treatments on intra and extracellular hemagglutinins from *Trichophyton rubrum* and *Trichophyton mentagrophytes*

Reagents	Titer			
	Trichophyton rubrum		*Trichophyton mentagrophytes*	
	Intra cellular	Extra cellular	Intra cellular	Extra cellular
Control	16	16	16	16
Trypsin (1%)	8	8	8	8
Na- Periodate (100mM)	4	4	4	4
Na- Citrate (100mM)	16	16	16	16
SDS (1%)	4	4	4	4
Deoxy Cholate (1%)	4	4	4	4
Urea (8M)	2	4	2	4
2-mercaptoethanol (400mM)	16	16	16	16

Table.4 Effect of divalent cations on intra and extracellular hemagglutinins from *T. rubrum* and *T. mentagrophytes*

Concentration of $CaCl_2$ (mM)	Titer[a]			
	Trichophyton rubrum		*Trichophyton mentagrophytes*	
	Intra cellular	Extra cellular	Intra cellular	Extra cellular
0	16	16	16	16
10	16	16	16	16
20	16	16	16	16
30	16	16	16	16
40	8	8	16	16
50	8	8	8	8
60	8	8	8	8
70	8	8	8	8
80	8	8	8	8
90	8	8	8	8
100	4	4	4	4

Acknowledgement

The authors are thankful to Professor A.K. Guha, (retd). Department of Biological Chemistry, Indian Association for the Cultivation of Science, Jadavpur, Kolkata-700032, India, for his work.

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