

## Significance of amino acid profile in the chemotaxonomic studies of keratinophilic fungi

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Presence or absence of certain amino acids and their ratio in a particular species of fungi are good subsidiary characters in distinguishing one species/genera from another (1-4). There is more variation in amino acid ratios at intergeneric level than at interspecific level (4). An attempt has been made, therefore, to study the amino acid profile of three keratinophilic fungi, namely, *Chrysosporium tropicum*, *Microsporium gypseum* and *Trichophyton mentagrophytes* and establish its role in chemotaxonomy. All the fungal isolates were isolated from soils of Jaipur using hair bait technique (5) and maintained on Sabouraud's Dextrose Agar. For this study, the fungi were grown in Sabouraud's broth for 10 days at  $28 \pm 2^\circ\text{C}$  and mycelium was harvested on the 11th day using Whatman filter paper no. 1 and washed with distilled water several times and these filter papers were oven dried at  $60^\circ\text{C}$  for 48 hours (6). The free amino acids were obtained using the known amount of mycelium by extracting in 80% ethanol. The bound amino acids were obtained using the known amount of mycelium with 6N HCl at 15 lbs for 30 minutes. The amino acids were analysed by Paper chromatography (Whatman no. 41) using Butanol-acetic acid-water (4:1:5 v/v) as running solvent and 0.1% Ninhydrin as developing reagent (4). The different amino acids and their ratios are summarised in Table-I. For identification of unknown amino acids, standard amino acids were run side by side with the unknown amino acids. The Rf values were calculated as :

$$\frac{\text{Total distance run by solute}}{\text{Total distance run by solvent}}$$

These were compared with Rf values of known amino acids and identified. Quantitative estimation of free and bound amino acids was done using a known

quantity of mycelium (500 mg). Standard Glycine graph was plotted using different concentration of Glycine (6). Optical density of free and bound amino acids of all the three test fungi was measured by Bosch and Lomb Spectrophotometer at 570nm. These were compared with the Standard Glycine Graph to get the concentration in  $\mu\text{g/g}$ . Rest of the procedure is same as in Parmar *et al.* (4). Of the 20 amino acids detected, *Microsporium gypseum* contained histidine, valine aspartic acid, isoleucine, serine and threonine in its free form and asparagine, alanine, valine, aspartic acid, isoleucine, tryptophan, arginine and lysine in its bound form. The ratio of free to bound amino acid was found to be 1:1.63. Aspartic acid was in highest concentration among all. In *Chrysosporium tropicum*, threonine, tryptophan, arginine and glutamic acid were lacking both in free and bound form but glycine was found in abundance as in *Microsporium gypseum*. *T. mentagrophytes* lacked glycine, leucine, cystine, and cystiene. The ratio of free to bound amino acids were 1:4.5 and 2.07:1 in *C. tropicum* and *T. mentagrophytes*, a striking dissimilarity was found in the ratios of all the three keratinophilic fungi. *C. tropicum* showed a major difference in the bound amino acid pattern also as compared to the other isolates, thereby indicating a distinct genetic makeup. The composition of mycelium, of fungi has been studied in recent years by a number of workers (4,8,10). Mathur and Sarbhoy (9) have already studied mycelial composition of *Sclerotium rolfsii* in detail. All the strains showed similar pattern.

During a study conducted (3-4), it was observed that isolates belonging to different genera had dissimilar pattern of amino acid and also their ratios. Present study also shows ample differences in the ratios of all the three fungi and also the pattern of free and bound amino acids. These observations are substantiated by morphological, cultural and microscopic studies. These findings are supported by Parmar *et al* (4) and Mathur and Sarbhoy (7).

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**Table 1.** Amino acid composition and its ratios in three keratinophilic fungi ( $\mu\text{g/g}$ )

Amino acid (Rf Value)	<i>M. gypseum</i>		<i>C. tropicum</i>		<i>T. mentagrophytes</i>	
	free	bound	free	bound	free	bound
Histidine (2)	37.5	-	-	145.0	-	37.5
Asparagine (35)	-	50.0	-	230.0	25.0	-
Glycine (19)	-	-	40.0	200.0	-	-
Alanine (36.3)	-	25.0	-	135.0	-	-
Proline (34.5)	-	-	-	55.0	-	2.5
Valine (65.4)	20.0	10.0	25.0	75.0	20.0	20.0
Aspartic acid (7.2)	32.5	37.5	20.0	-	35.0	30.0
Tyrosine (30)	-	-	45.0	-	-	2.40
Isoleucine (87.2)	25.0	25.0	20.0	-	2.5	21.8
L-Leucine (19)	-	-	25.0	-	-	-
Cystine (77)	-	-	-	-	-	-
Serine (18.5)	25.0	-	-	-	-	-
DL-Threonine (26)	25.0	-	-	-	-	-
Tryptophan (65.4)	-	20.0	-	-	10.0	15.0
Arginine (14.5)	-	37.5	-	-	187.0	-
Glutamic acid (28)	-	-	-	-	50.0	-
Ornithin (14.5)	-	-	-	-	-	20.0
Methionine (21)	-	-	-	-	25.0	-
Lysine (11.5)	-	25.0	-	-	37.5	-
Cysteine (24)	-	-	-	-	-	-
Unidentified (9)	-	62.5	10.0	-	-	-
Total amino acid content	142.5	292.5	185.0	840.0	367.0	176.2
Total of free and bound amino acids	435.0		1025.0	543.2		
Free: Bound amino acids	1:1.63		1:4.54		2.07:1	

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## REFERENCES

1. Agnihotri, V.P. and Mehrotra, B.S. (1961). *Lloydia* **24**: 41-44.
2. Sarbhoy, A.K. (1963). *Mycopath. Mycol. appl.* **19**: 37-43.
3. Bhatnagar, R.K. and Mukerjee, K.G. (1978). *Indian Phytopath.* **31**: 374-375.
4. Parmar, S.M.S., Williamson, Taneja, S. and Joshi, M. (1984). *Indian Phytopath.* **37**(3): 446-448.
5. Vanbreuseghem, R. (1952). *Ann. Soc. Belge. Med. Trop.* **32**: 173-178.
6. Sadasivam, S. and Manickam, A. (1993). In: *Biochemical Methods*, Wiley Eastern Ltd. pp. 216
7. Mathur, S.B. and Sarbhoy, A.K. (1978). *Indian Phytopath.* **31**: 114-115.
8. Venkataraman, C.S. (1956). *Proc. Natl. Ist. Sci.* **22**: 227-236.
9. Mathur, S.B. and Sarbhoy, A.K. (1977). *Indian Phytopath.* **30**: 294-295.
10. Kushwaha, R.K.S. (1984). *Proc. Indian Nat. Sci. Acad.-B- Biological Sciences.* **50**: 218-222.

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