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Comparative analysis of colonization and perforation of human hair by different strain of *Chrysosporium tropicum* growing on human hair in sand culture over a certain time period

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Abstract
This study is based on the preliminary test on some strains of most viable Keratinofilic fungi *C. tropicum* growth and performance on human hair perforation and degradation by the colonization. Members of the genus *Chrysosporium* are related to the dermatophytes but information on penetration of hair by the different strains of *C. tropicum* has not yet been reported. The present work investigate the hair perforating ability of different strains of *C. tropicum*, to select: out most efficient strains among them for further detailed studies.

Keywords: *Chrysosporium*, hair perforating ability, keratinofilic fungi

Introduction
The investigation on the keratinolysis involves three basic methodological approaches morphological, physiological and chemical. Morphological changes during keratin decomposition were studied by English (1963, 1968, 1976) and Vanbreuseghem (1949, 1952) [20, 21]. Davidson and Gregory (1934) [22] were among the first who described the formation of wedge shaped perforations in hair exposed to the direct effect of T. mentagrophytes. Vanbreuseghem (1949, 1952) [20, 21] observed and described different mode of attacking the hair by different species of dermatophytes. He reported two basic types of decomposition and in accordance with it he divided dermatophytes into two groups. The first group represented by T. mentagrophytes which developed typical perforation vertically to the longitudinal axis of hair while the second group, represented by T. Rubrum and caused flat erosions of the hair surface. Page (1950) described perforation organs in M. gypsum and termed them as "Intrusions". Daniels (1953) [23] studied the course of decomposition in M. Canis and noticed particular "Frond Like" formations which he suggested to be responsible for the cuticle lifting and decomposition of the hair.
Mercer and Verma (1963) [24] studied the invasion of sterile human hair by the fungus T. mentagrophytes in a humid chamber. They found all characteristics of enzymatic breakdown. In the cuticle as well as in the cortex the fungus grew at first intercellulary. Later on hyphae directly penetrating the cells could be observed. In the hair cuticle the internal cell layer (the endocuticle) was disgusted while more resistant layers (The executable and epicuticle) remained intact after five days growth of the fungus on the hair. In the cortex the decomposition manifested itself by the separation of bundles of keratin fibrils and by their gradual disintegration. In the most affected sites complete disappearance of the bundles was observed by Mercer and Verma (1963) [24], Baxter and Mann (1969) [25] examined human hair invaded by T. mentagrophytes, T. Ajelloi and T. Rubrum. The character of degradation described 'corresponded to the findings of the Mercer and Verma (1963) [24], however, the degree of decomposition was comparatively low. Clear signs of lies were found in case of T. mentagrophytes only. In T. Ajelloi and T. Rubrum an intercellular growth was typical and the hyaE were often accumulated among obviously intact elements of both the cuticle and cortex. Mercer (1961) [26] provides a standard reference on keratin and its synthesis. Good summaries of various aspects of the properties and degradation have appeared (De Vries, 1962 [27]; Chesters and Mathison, 1963 [28] and English 1965).
The ability of keratinophilic fungi to attack and perforate hair *in vitro* has been considered to be restricted to dermatophytes and related fungi (Davidson and Gregory, 1934 [22]; Page 1960; Daniels, 1953 [29]; Barlow and Chatta Way, 1955 [30]; Ajello and Georg, 1957 [31];

Carmichael, 1962 [32]; Lu, 1962) [33]. Members of the genus *Chrysosporium* are related to the dermatophytes but information on penetration of hair by the different strains of *C. tropicum* has not yet been reported. The present work investigate the hair perforating ability of different strains of *C. tropicum*, to select: out most efficient strains among them for further detailed studies.

Materials and Methods

The test procedure followed was that of Ajello and Georg (1957) [34]. Fifteen isolates of *C. tropicum* were examined for their colonized and perforating ability of human hair.

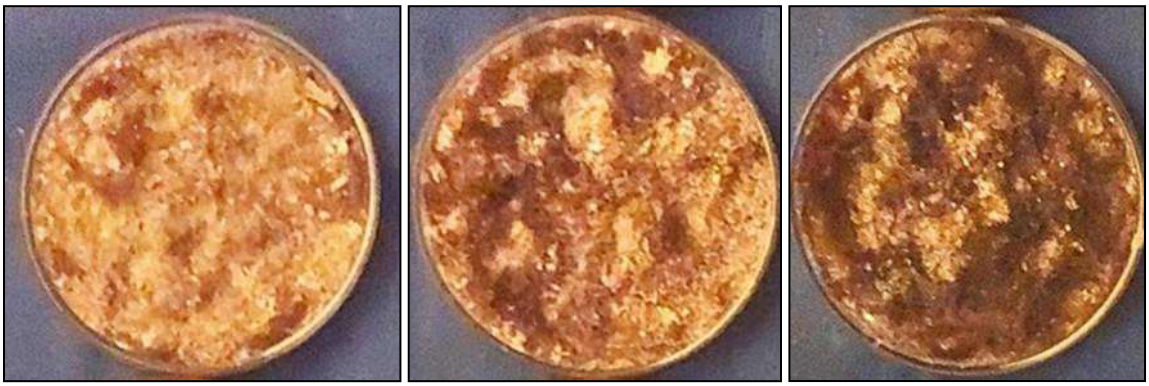
Several fragments of colonies of *C. tropicum* served as inoculum which were taken from 7 days old colonies. Black hair of young women were cut into small pieces. Sterilized human hair segments were placed in petrifies to which thirty grams of washed and sterilized sand was kept (Plate 1-2). The sand was moistened by adding sterilized water. The dishes were examined daily for any sign of mycelial growth on hair. The microscopic observations, were made at 7 days interval over a period of 42 days.

Hair segments overgrown with mycelium were removed from the petrifies after each 7 days of incubation with sterile forceps, placed in a drop of lacto phenol cotton blue mounting fluid and examined under the microscope for hair perforation and micro morphological changes in the hair caused by the test fungi. The hair segments were considered to be lysed when they could not be picked up with the help of forceps and the bundles of keratin fibrils proceeded to separation and disintegrate hair segments showed faded colour when comparing with control. The experiments were conducted at 28:2 °C in the dark. All the experiments were carried out in triplicate.

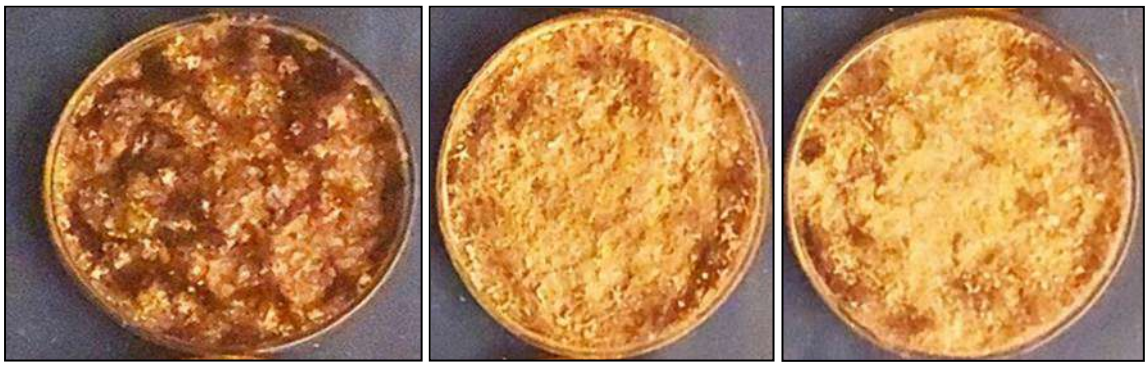
The percent hair perforation was calculated as follows: Per cent hair Perforation = Number of hair segments perforated / Total number of hair segments X 100.

C. tropicum GPCK 511 *C. tropicum* GPCK 510 *C. tropicum* GPCK 512

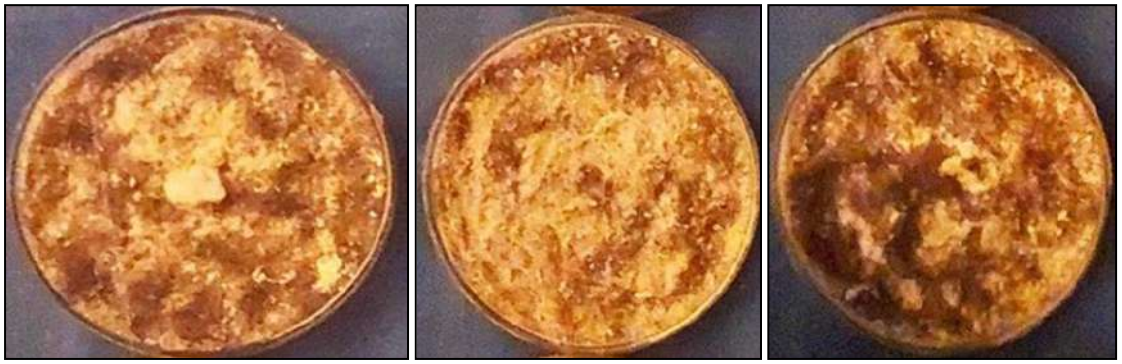
Plate 1: Three different strains of *Chrysosporium* tropicum growing human hair in sand culture at 42 days of incubation period



AC tropicum GPCK 516 *C. tropicum* GPCK 521 *C. tropicum* GPCK 517



BC tropicum GPCK 518 *C. tropicum* GPCK 520 *C. tropicum* GPCK 519



CC tropicum GPCK 524 *C. tropicum* GPCK 515 *C. tropicum* GPCK 523

DC tropicum GPCK 513 *C. tropicum* GPCK 522 *C. tropicum* GPCK 514

Plate 2: Different strains of *Chrysosporium* tropicum growing on the hair in sand culture at 42 days of incubation period

Table 1: Colonization and perforation of human hair by different strain of *Chrysosporium*

Different strains of Chrysosporium tropicum	Time taken for colonization (Days)	Perforation Days (%)					
		7	14	21	28	35	42
C. tropicum GPCK 510	16	0	31.6±2.8	43.3±2.8	58.3±7.6	80.0±5.0	93.0±2.8
C. tropicum GPCK 511	7	0	63.3±5.7	70.0±5.0	70.0±0.0	100.0±0.0	100.0±0.0
C. tropicum GPCK 512	7	0	60.0±0.0	63.0±5.7	91.0±0.0	100.0±0.0	100.0±0.0
C. tropicum GPCK 513	12	0	30.0±0.0	48.3±2.8	61.6±2.8	63.3±2.8	75.0±0.0
C. tropicum GPCK 514	24	0	21.6±2.8	31.6±2.8	50.0±5.0	60.0±0.0	65.0±0.0
C. tropicum GPCK 515	17	0	28.3±2.8	61.6±5.7	71.6±2.6	80.0±0.0	80.0±0.0
C. tropicum GPCK 516	7	0	46.6±5.7	56.6±5.7	63.3±2.8	90.0±0.0	90.0±0.0
C. tropicum GPCK 517	32	0	40.0±0.0	50.0±0.0	55.0±0.0	63.0±0.0	73.3±2.8
C. tropicum GPCK 518	32	0	48.0±2.8	55.0±8.6	63.3±2.8	73.0±2.8	80.0±0.0
C. tropicum GPCK 519	30	0	41.6±2.8	51.6±5.7	66.6±5.7	80.0±0.0	85.0±0.0
C. tropicum GPCK 520	18	0	30.0±5.0	43.3±2.8	53.3±2.8	58.3±7.6	75.0±0.0
C. tropicum GPCK 521	40	0	30.0±5.0	40.0±5.5	51.6±2.8	53.3±2.8	58.3±2.8
C. tropicum GPCK 522	0	0	0.0	0.0	0.0	0.0	0.0
C. tropicum GPCK 523	12	0	31.6±5.7	55.0±2.8	61.6±2.8	70.0±0.0	70.0±0.0
C. tropicum GPCK 524	20	0	20.0±5.0	23.0±2.8	40.0±0.0	43.4±2.8	60.0±0.0

Results and Discussion

The results of human hair perforations are summarized in Tables 1-2 and Fig. 1. The microscopic observation of the hair segments revealed that *C. tropicum* GPCK 511 and *C. tropicum* GPCK 512 formed colonies on the hair in seven days while *C. tropicum* GPCK 523, GPCK 513 required twelve days, whereas, *C. tropicum* GPCK 510, GPCK 520 and GPCK 515 took sixteen to eighteen days to grow and form colonies on human hair. *C. tropicum* GPCK 521, GPCK 518, GPCK 517, GPCK 514 and GPCK 519 took 24-40 days to colonize hair. The best perforator was found to be ci tropicum GPCK 511, and GPCK 512 as they digested hair within thirty five days. These were closely 'followed by *C. tropicum* GPCK 510, GPCK 516, GPCK 515, and *C. tropicum* GPCK 519, *C. tropicum* GPCK 512 showed sixty per cent perforation of hair in 14 days. In 21, 28, 35 and 42 days the hair perforation was increased to 63.0, 91.0, 100 and 100 percent respectively. The maximum rate of hair perforation in *C. tropicum* GPCK 510 was recorded to be 93.0 per cent, in 42 days of incubation. The rate of

perforation of human hair in 14, 21, 28 and 35 days by the same strain was 31.6, 43.3 and 58.3 and 80.0 per cent respectively. *C. tropicum* GPCK 515 showed 28.3 61.6, 71.6, 80.0 and 80.0 per cent perforation of hair different incubation periods of 21 to 42 days.

1. *Chrysosporium* tropicum GPCK 510
2. *Chrysosporium* tropicum GPCK 511
3. *Chrysosporium* tropicum GPCK 512
4. *Chrysosporium* tropicum GPCK 513
5. *Chrysosporium* tropicum GPCK 514
6. *Chrysosporium* tropicum GPCK 515
7. *Chrysosporium* tropicum GPCK 516
8. *Chrysosporium* tropicum GPCK 517
9. *Chrysosporium* tropicum GPCK 518
10. *Chrysosporium* tropicum GPCK 519
11. *Chrysosporium* tropicum GPCK 520
12. *Chrysosporium* tropicum GPCK 521
13. *Chrysosporium* tropicum GPCK 522
14. *Chrysosporium* tropicum GPCK 523
15. *Chrysosporium* tropicum GPCK 524

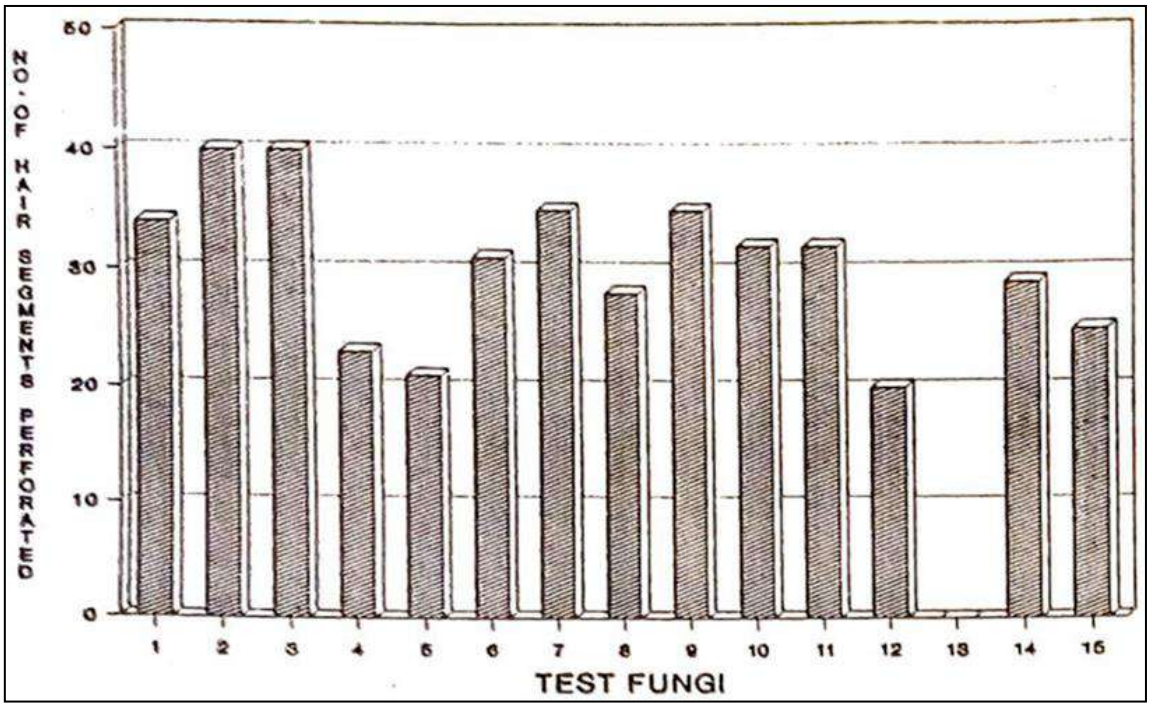


Fig 1: Number of hair segments perforated by different stains of *Chrysosporium* tropicum

Table 2: Comparative keratinophilic ability of different strains of *Chrysosporium* tropicum

Different strains of Chrysosporium tropicum	Minimum value of hair perforation	Maximum value of hair perforation (%)	Incubation Period (Days)
C. tropicum GPCK 511	63.3 ± 5.7	100.0 ± 0.0	35
C. tropicum GPCK 512	60.0 ± 0.0	100.0 ± 0.0	35
C. tropicum GPCK 510	31.6 ± 2.8	93.0 ± 2.8	42
C. tropicum GPCK 516	46.6 ± 5.7	90.0 ± 0.0	35
C. tropicum GPCK 519	41.6 ± 2.8	85.0 ± 0.0	42
C. tropicum GPCK 518	48.0 ± 2.8	80.0 ± 0.0	42
C. tropicum GPCK 515	28.3 ± 2.8	80.0 ± 0.0	35
C. tropicum GPCK 513	30.0 ± 0.0	75.0 ± 0.0	42
C. tropicum GPCK 520	30.0 ± 5.0	75.0 ± 0.0	42
C. tropicum GPCK 517	40.0 ± 0.0	73.3 ± 2.8	42
C. tropicum GPCK 523	31.6 ± 5.7	70.0 ± 0.0	35
C. tropicum GPCK 514	21.6 ± 2.8	65.0 ± 0.0	42
C. tropicum GPCK 524	20.0 ± 5.0	60.0 ± 0.0	42
C. tropicum GPCK 512	30.0 ± 5.0	58.3 ± 2.8	42
C. tropicum GPCK 522	0.0	0.0	42

C. tropicum GPCK 523 showed 31.6, 55.0, 61.6, 70.0 and 70.0 per cent perforation in different intervals. *C. tropicum* GPCK 513: showed 30.0, 48.3; 61.6, 63.3 and 75.0 percent perforation in 14, 21, 28, 35 and 42 days of incubation

period respectively. C tropicum GPCK 524 showed least hair perforation at different incubation periods as its perforation was found to be 20.0, 23.0, 40.0, 43.3 and 60.0 per cent only. *C. tropicum* GPCK 517 revealed 73.3 percent hair perforation at the end of incubation period. Lowest value of per cent in hair perforation was noticed at 14 days of incubation. *C. tropicum* GPCK 518 showed 48.0, 55.0, 63.3, 73.0 and 80.0 per cent" perforation in different periods of incubation from 14 to 42 days respectively. The level of hair perforation reached to the extent of 85.0 per cent in 42 days of incubation in the case of GPCK 519 strain. It was noted that marked increasing trend in hair perforation with the increase in days of incubation period and lowest value of 41.6 per cent was recorded at 14 days which was followed by 51.6. 66.6 And 80.0 per cent in 21, 28 and 35 days of incubation respectively. The strain GPCK 516 caused hair perforation up to 90 per cent towards the end of incubation period of 42 days whereas GPCK 521 caused 58.3 per cent hair perforation at the end of incubation period.

Conclusion

The performance of the strain GPCK 520 was comparatively better as values of 30.0, 43.3, 53.3, 58.3 and 75.0 per cent were recorded in respect of hair perforation at 14, 21, 28, 35 and 42 days respectively. In contrast to the above findings *C. tropicum* GPCK 522 did not show any keratinolytic activity till the end of 42 days. *C. tropicum* GPCK 514 also had the lower keratinolytic activity showing 65 per cent hair perforation in 42 days of incubation.

From the above study we can conclude that 14 strain of *C. tropicum* used to have an ability to degrade the human hair, the best performance was given by the strain of *C. tropicum* strain GPCK 511 and GPCK 512, it is therefore these two strain were selected for further study.

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