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ORIGINAL RESEARCH ARTICLE

Human Hair Perforating Ability of *Chrysosporium tropicum* Strains

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ABSTRACT

Fifteen isolates of *C. tropicum* isolated from soil were examined for their colonization and human hair perforating ability. On the basis of screening it was concluded that the 15 strains of *C. tropicum* used have got an ability to degrade hair. The best performance was given by two strains of *C. tropicum* i.e. GPCK 511 and GPCK 512.

Key words: *Chrysosporium*, human hair, perforation.

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, 1965, 1976) and Vanbreuseghem (1949, 1952). Davidson and Gregory (1934) were among the first who described the formation of wedge shaped perforations in hair exposed to the direct effect of *T. mentagrphytes.* Vanbreuseghem (1949, 1952) observed and described different mode of attacking the hair by different species of of dermatophytes. He reported two basic types of decomposition and in accoradance 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. gypseum* and termed them as “Intrusions”. Daniels (1969) 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) 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 intercellular. Later on hyphae directly penetrating the cells could be observed. In the hair cuticle the internal cell layer (the endocuticle) was desgested while more resistant layers (the exocuticle 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 their gradual disintegration the most affected sites complete disappearance of the bundles was observed by Mercer and Verma (1963). Baxter and Mann (1969) 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), however, the degree of the decomposition was comparatively low. Curling signs of lysis were found in case of *T. mentagrophytes* only. In *T. ajelloi* and *T. rubrum* an intercellular growth was typical and the hypae were often accumulated among obviously intact elements of both the cuticle and cortex. Mercer (1961) provides a standard reference on keratin and its synthesis. Good summaries of various aspects of the properties and degradation have appeared (Chesters and Mathison, 1963 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; Page 1960; Barlow and Chattaway, 1955; Ajello and Georg, 1957; Lu, 1962). Members of the genus *Chrysosporium* are related to the dematophytes but information on penetration of hair by the different strains of *C.*

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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 strans among them for further detailed studies.

MATERIALS AND METHODS

The test procedure followed was that of Ajello and Georg(1957). 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 inoculums which were taken from 7 days old colonies. Black hairs of young women were cut into small pieces. Sterilized human hair segments were placed in petridishes to which thirty grams of washed and sterilized sand was kept. The sand was moistened by adding sterilized water. The dishes were examined daily for any sign of mycelia 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 petridishes 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 color when comparing with control. The experiments were conducted at 28 ± 2^{o}C in the dark. All the experiments were carried out in triplicate.

The per cent hair perforation was calculated as follows:

% hair perforation= No. of hair segments perforated
----------X100
Total No. of hair segments

RESULTS AND DISCUSSION

The results of human hair perforations are summaries in (**Table 1 & 2**). 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 510, GPCK 520 and GPCK 515 took sixteen to eighteen days to grow and form colonies on human hair. *C. tripicum* 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 *C. 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. tropium* 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 per cent respectively. The maximum rate of hair perforation in *C. tropicum* GPCK 510 was recorded to be 93.0 percent, 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 per cent perforation of hair in different incubation periods of 21 to 42 days.

C. tropicum GPCK 523 showed 31.6, 55.0, 61.6 70.0 and 70.0 percent perforation in different intervals, *C. tropicum* and GPCK 513 showed 30.0, 48.3, 61.6, 63.3 and 75.0 per cent respectively *C. tropicum* GPCK 524 showed least hair perforation 20.0, 23.0, 40.0, 43.3 and 60.0 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 percent 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 percent 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.

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 percent hair perforation in 42 days of incubation. 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.

The comparative performance of different *C. tropicum* strains in respect of their ability to perforate the human hair is illustrated in Table 2 along with range of hair perforation at screened for their kerationophilic activity the strain GPCK 511 and GPCK 512 were found to be highly keratinolytic in nature as cent within 35 days of

incubation. These strains were followed by GPCK 510, GPCK 519 as they were also highly effective in perforating the hair up to the level of 93.0, 90.0 and 85.0 per cent in 42 days of incubation respectively. It was interesting to note that *C.tropicum* GPCK 522 did not colonize human hair even after 42 days of incubation. The strain GPCK 521 and GPCK 524 were of low keratinolytic ability as these strains could perforate the human hair to the extent of 58.3 and 60.0 per cent respectively in maximum period of incubation i.e. 42 days.

In a related type of study Padhye *et. al*., (1980), have carried out in vitro experiments on the ability to perforate hair of 44 species of different dermatophytes. It was observed that the ability to perforate hair is specific character of that fungus which did not show variation among the isolates of species. However, experiments conducted by the author on fifteen strains, of *C. tropicum* it was found that fourteen strains possessed ability to perforate the hair, one strain did not possess perforating ability.

During perforation study different types of micro morphological changes were observed in hair. Cuticle, cortex and medulla are three parts of the hair. Tuft of mycelium and conidial germination on hair surface has been frequently observed. In some hair segments cuticle undulation and cortex disruption was noted. Perforating organs were found in cortex and medulla region. These perforating organs were of different shape and size. Perforators were narrow, broad, short and long. When the perforator was less then the half of width of hair, it was supposed to be short and when it was more than half of width of hair then it was regarded to be long. Shortest perforator measured to um and longest one was 91. um. Different shapes of perforators like spoon-shaped, needle shaped torpedo-shape, pin-head shaped, fissure-like, tunnel like and wedge shaped. Cortical perforation and wedge shaped perforation were observed in hair colonized by *C. tropicum* GPCK 511, Cuticle lifting, modularly perforation and cuticle disruption caused by *C. tropicum* GPCK 510 and GPCK 515 were found. Pin-head shaped perforation, medullary digestion of hair cuticle disruption modularly perforation were noted in hair penetrated by *C. tropicum* GPCK 511, GPCK 519 and GPCK 518 . Neddle shaped perforation were observed in case of *C. tropicum* GPCK 511, GPCK 512 and GPCK 516, Spoon shaped perforators were formed in *C. tropicum* GPCK 514 and GPCK 517. Tunnel and fissure shaped perforator was observed in *C. tropicum* GPCK 511, GPCK 513, GPCK 520 and GPCK 519. Torpedo shaped, fissure shape perforators and cuticle disruption were observed in *C. tropicum* GPCK 512, GPCK 511 and GPCK 516. Medullary perforation and modularly digestion were also observed in *C. tropicum* GPCK 511 and GPCK 512 (Plate 6). Complete digestion of hair has noted within 35 days of incubation in case of strain GPCK 511 and GPCK 512, hair segments were also 1ysed and decolorized in both the strains. No changes have been observed in hair and fungal controls.

In a study by Masmuto *et. al* (1983), the isolates of *T. tonsurans* var.*sulfureum* fell into two groups – perforators and non-perforators. Six of 35 isolate were found to perforate hair in contrast to the remaining 29. The growth of fungi on hair without perforating organs may be due to their utilization of non-keratinous substrates present in hair (Safranek and Goss (1982).

Evan and Hose (1975) classified isolates of *Hendersonula toruloidea* into three categories on the basis of to breakdown hair, group B little or no evidence of breakdown ability and group-C with an intermediate results. Ajello and Georg (1957) found wedge shaped perforation in *T. mentagrophytes* penetrated hair segments. Otcenesek and Dvorak (1964) used the word ‘penetrating organs, for the perforators. English (1963) observed cuticle lifting, erosion of cortex and perforation organs in hair penetrated by C. keratinophilum Torpedo shaped perforators were reported by Shrivastava (1985) and Jonifer et. al., (1989) observed tunnels fissures in hair penetrated by soil fungi. Tunnel formation by *H. toruloida* in hair was reported by Campbell (1974). Evolceanui and Maria (1960) used the expression ‘organs for perforators. Shallow depressions and perforation canals were cuticle disruption, cuticle undulation, narrow perforating organs, and complete digestion of hair has been observed by Bahuguna.and Kushwaha (1989). Species attacked hair was found to be intermediate between the dermatophytes and non-kerationophilic molds in the present study. The keratinolytic ability of some *Chrysosporium* spp. was similar to that of dermatophytes (Kushwaha, 1983).

On the basis of this preliminary screening it was concluded that the 15 strains of *C. tropicum* used have got an ability to degrade hair. The best performance was given by two strains of *C. tropicum* i.e. GPCK 511 and GPCK 512; it is

therefore these two strains were used for further study.

Table 1: Colonization and perforation of hair by different strains of *Chrysosporium tropicum*

Different strains of *Chrysosportum tropicum*	Time taken for colonization (Days)	Perfoat1on (%)						Micromorphological changes in hair
		7	14	21	28	35	42	
C. tropicum GPCK 510	16	0	31.6±2.8	43.3±2.	58.3±7.6	80.0±5.0	93.0±2.8	CL, CD; MP, L,WP,MO
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.	NL, MP; MO,SP, NPO,CD, CP,FS,TS,WP,PHS
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	MP, NPO,CD,CP,CU,WP, FS,NL, TP ,MO
C. tropicum GPCK 513	12	0	30.0±0.0	48.3±2.8	61.6±2.8	63.6±5.8	75.0.0±0	TP, FS
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	CL, NL, SP
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	CD, CL, MP
C. tropicum GPCK 516	7	0	46.6±5.7	56.6±2.7	63.3±2.8	90.0±0.0	90.0±0.0	NL,SP,TP,FS,CO
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	MP, CL, SP
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	MP,PHS,MD,CO,
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	TP, FP, HL,PHS,MO, CD MP
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	MP ,CL,NL, TP, FP
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	NL, SL
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	NL, CL
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	MP, CL, NL.

CL - Cuticle Lifting, CD - Corticle Disruption, MP - Medullary Perforation, HL - Hair Lysed, WP - Wedge Perforation, MO - Medullary Digestion, NL - Needle Like Perforation, SP - Spoon Shaped Perforation, NPO - Narrow Perforation Organ, CP, Cortical Perforation, FS - Fissure Shaped, TS - Tunnel Shaped, PHS - Pin Head Shaped, CU - Cuticle Undulation, TP - Torpedo Shaped Perforation.

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

Different strains of *Chrysosportum 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	35
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

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REFERENCES

1. Ajello, L. and Georg. L.K. 1957. *In vitro* hair cultures for differentiating between atypical isolates of *Trichophyton rubrum*. Mycopath. Mycol. Appl. 8: 3-17.
2. Bahuguna, S. and Kushwaha, R.K.S. 1989. Hair perforation by keratinophilic fungi. Mykosen. 32 : 340-343.
3. Barlow, A.J.E. and Chattaway, F.W. 1955. The attack of chemically modified keratin by certain dermatophytes. J. Invest. Dermatol. 24: 65-75.
4. Baxter, M. and Mann, P.R. 1969. Electron microscopic studies of the invasion of human hair invitro by three keratinophilic fung. Sabouraudia. 7: 33-37.
5. Campbell, C.K. 191974. Studies on *Hendrsonula toruloidea* isolated from human skin and nail. Sabouraudia. 12: 150-156.
6. Chesters, C.G.C. and Mathison, C.E. 1963. The decomposition of wool keratin by *Keratinomyces ajelloi.* Sabouraudia. 2: 225-237.
7. Davidson, A. M. and Gregory, P .H. 1934. *In situ* cultures of dermatophytes. Can. J. Res. 10: 373-393.
8. Daniels. G. 1953. The digestion of human hair keratin by *Microsporum canis*. J. Gen. Microbial. 8: 289-294.
9. De Vries. 1962. Keratinophilic fungi and their action. Antonie Van

Leeuwenhock.28: 121-133.
10. English, M. P. 1963. The saprophytic growth of keratinophilic fungi on keratin. Sabouraudia. 2: 115-130.
11. English, M. P. 1965. The saprophytic growth of non-keratinophilic fungi on keratinized substrate and a comparison with keratinophilic fimgi. Trans. Brit. Mycol. Soc. 48: 21.9-235.
12. English, M.P. 1976. Destruction of hair by two species of Chrysosporium trans. Brit. Mycol. Soc. 66: 357-358.
13. Evans, E.G.V. and Hose. H. 1975. In vitro degradation of human hair by *Hendersonula toruloidea*. Subouraudla. 13: 323-328.
14. Evolceanui, R. and Maria. L. 1960. Le problem de l' existence et de lamise in evidence des ferments keratinolytiques chez leas dermatophytes. Mycopath. Mycol. App. 12: 216-222.
15. Jonifer, A., Kundrat and Rowe t F. 1989. Study of hair degradation in agricultural soil. Biodeterioration Research. 2: 91-98.
16. Kushwaha. R.K.S. 1983. The in vitro degradation of peacock feathers by some fugi. Mykosen. 26: 324-326.
17. Lu, Y. C. 1962. A new method for the study of hair digestion by dermatophytes. Mycopath. Mycol. Appl. 17: 225-235.
18. Matsmuto, T. Padhya, A.A. and Ajello, L. 1983. In vitro hair perforation by a new subvariety of *Trichophyton tonsurans* var, *sulfureum*. Mycotaxon. 28: 235-242.
19. Mercer, E.H. 1961. Keratin and keratinization. An Ssa Pesgam on Press Oxford.
20. Mercer. E. H. and Verma. B. S. 1963. Hair digested by *Trichophyton mentagrophytes*. J. Invest. Dermatol. 48: 357-360.
21. Otcenasek, M. and Dvorak. J. 1964. The Isolation of *Chrysosporium keratinophilum* (Frey) Carmichael and similar ftmgi frnm Czechoslovakian soil. Mycopath. Mycol. Appl. 23: 121-124.
22. Padhye, A.A., Young C.N. and Ajello, L. 1980. Hair perforation as a diagnostic criterian in the indentification of *Epidermophyton*, *Microsporum* and *Trichophyton* species. In superficial cutaneous and subcutaneous infections. Pong American Health Organization Sci. Publ. No. 396. Washinghton. D.C. 115-120.
23. Page, R.M. 1950. Observations on keratin digestion by *Microsporum gypseum*. Mycologia. 42: 591-602. Press Oxford.
24. Safranek. W.W. and Goos, R.D. 1982. Degradation of wool by saprophytic fungi. Can. J. Microbial. 28: 138-140.
25. Shrivastava, J. N. 1985. Studies on soil inhabiting dermatophytes and keratinophilic fungi. Ph.D. Thesis Kanpur University. Kanpur. 254.
26. Vanbreuseghem, R. 1952. Keratin digestion by dermatophytes a specific diagnostic method. Mycologia. 44: 176-182.
27. Vanbreuseghem, R. 1949. La cultures des dermataphytes in vitro. Sur de cheveux isoles Ann. Parasitol 24: 559-573.