Int J Pharm Bio Sci 2013 July; 4(3): (B) 1373 - 1380

Research Article **Microbiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

OPTIMIZATION OF KERATIN DECOMPOSITION BY *CHRYSOSPORIUM TROPICUM*

FIRDOS KATIAR AND R.K.S.KUSHWAHA*

Department of Botany, Christ Church College, Kanpur 208 001 Firdos. katiar@gmail.com

ABSTRACT

The loss in weight of keratin substances, pH change and protein released into the culture medium provide definite evidence of the proteolytic and keratin decomposing ability of *C. tropicum* GPCK 511 and *C. tropicum* GPCK 512. However, the results showed that more protein released and more weight loss was found in shaking condition in comparison to static condition. From the results it is clear that maximum values of protein and weight loss were mostly obtained at 10 to 15 days indicating optimum incubation at this period.

KEY WORDS: ***Chrysosporium*. keratin decomposition.**

R.K.S.KUSHWAHA
Shri Shakti College, Ghatampur, Kanpur 209 206,
kushwaharks@gmail.com

INTRODUCTION

It has been observed that the aspect of keratin decomposition has been dealt under physiological and biochemical aspects. The paper concerning this problem from a deeper physiological and biochemical stand point were published by Stahl et. al (1949, 1950). Later on many other papers appeared where the authors studied the submerged cultures of dermatophytes on keratinous substrates (Kunert, 1972, 1973, 1981; Mathison, 1965; Chester and Mathison, 1963, Ragot, 1966, 1967, 1968; Weary et. al 1965; Ersty snf Vsnny, 1967, 1969' Evans and Hose 1975; Safranek and Goos 1982 ; Kushwaha 1983). The investigations on keratin decomposition proved that keratinophilic fungi can grow even on very hard keratin as the only sources of carbon and nitrogen. Decomposition is accompanied by alkalization of medium and by the high activity of proteolytic exoenzyation of the medium and by the high activity of protelytic exoenzymes. Evolceanui and Lazar (1960) first suggested the presence of extra cellualar keratinolytic enzyme in dermatophytes and found amino acids as the breakdown product of keratain. Kunert (1972) found the products of culture filtrate with characters of aminoacids, peptides and proteins. Nickerson and Durand (1963), Peyton et al (1965) and Ziegler (1967) clearly indicated that these dermatophytes are well equipped with enzymes able to provide them organic nutrients highly resistant keratinous substrates. The keratinases of keratinophilic fungi and especially dermatophytes has attracted attention in recent years as these enzymes are almost associated with mycotic infection of human and other animals Yu et al (1968, 1969, 1972) isolated extracellular keratinases and studied their role in substrate decomposition. Singh and Agarwal (1987) observed the role of proteolytic enzymes produced by certain keratinophilic fungi in keratin decomposition.

Some plant pathogenic dematiaceous fungi (Evans and Hose, 1975), Saprophytic fungi (Sfranek and Goos. 1982) and geophilic fungi (Kushwaha 1983) were evidently consumed human hair, wool and peacock feathers. These digest keratin by means of a keratinolytic system that includes active alkalization of the substrates, the extra celluar suflitlysis of disulfide bonds and proteolysis of keratin molecules. Such evidences are not available for hyphomycetous *Chrysosporium*. More ever its ability to utilized keratin as sole source of nutrients has not yet been established. More ever, while working on the decomposition of keratinous substrate it becomes 1mperative to set up reaction conditions to achieve successfully meaningful results. Different fungi vary for maximum decomposition of keratin also varies. In order to calibrate incubation period required to decompose human hair the two strains of *C. tropicum* i.e. *C. tropicum* GPCK 511 and *C. tropicum* GPCK 512 were selected in the present screening trial.

MATERIALS AND METHODS

Human hair of the persons of age ranging 16-25 years were obtained and brought to the laboratory. These were cut in 2 cm. long pieces and washed 5 times with sterilized distilled water and air dried. Preliminary examination revealed negligible le protein release. The hair was then weighed into 200 mg. aliquots. The basal medium contained the following ingredients per liter of glass distill3d water : K_2HPO_4_1.0 gm; $MgSO_4$. $7H_2$o-0.5 gm; KCl-0.5 gm $NaNo_3$-2.0 gm; $FeSO_4.7H_2O$-0.01 gm and sucrose-30 gm. Two hundred fifty ml Erlenmeyer flasks containing 50 ml basal medium and 200 mg. human hair were autoclaved at 15 1bs pressure for 10 minutes. The protein present in the medium was subtracted. The spore suspension was obtained from the surface of 6 days old cultures previously grown on mineral medium by brushing spores in 5 ml of sterilized distilled water. The following three control flasks were run :

1. Keratin control to which were added 50 ml of mineral medium and 200 mg of hair (human).
2. Fungus control to which were added 50 mal of mineral medium and fungal inoculums.
3. Test sample to which were added 50 ml of mineral medium and 200 mg of hair and fungal inoculums.

The flasks were incubated as static and shaking conditions at 28±2^{o}C and filtered after different incubation periods i.e. 5, 10, 15, 20 and 25 days in order to optimize the time of incubation for protein released in the medium

UTILIZATION OF KERATIN

The utilization of hair keratin was assessed by following method after different incubation periods. Substrate was removed and dried at 80^{o}C. The filtrates of keratin controls, fungal control and test samples were obtained by filtration and tested for protein released. The substrates were neither washed nor the mycelium was removed. The dry weight of one wad of hair at the end of incubation period was compared to its initial weight (200 mg.) If substrate plus mycelium weight more than at the beginning the fungus was considered to be non-keratinolytic and the increased weight was considered to be non keratinolytic and the increased weight was attributed to the mycelia production utilizing the nutrients from the liquid medium (Fergus 1969). It was recorded as zero loss of weight. If the substrate weighed less than at the beginning, the weight loss was calculated and then the average of the weight of the inoculated keratin substrate was substituted. If there was still a weight loss the fungus was considered keratinolytic and the net loss attributed to the loss of non-recoverable products of keratin degradation. Results were presented as zero loss if positive values were obtained.

PROTEIN DETERMINATION

For this experiment human hair was used as substrate. The degradation of hair was monitored by determination of protein released into the medium. Samples were taken from each flask after desired incubation period. Culture filtrate from each flask was centrifuged at 4000 rpm for 5 minutes and the supernatant was assayed for protein by using Folin-Ciocalteu regent (Lowry, et al., 1951; Packer 1967) as described below :

Preparation of alkaline reagent

Solution I : Two percent Na_2Co_3 in 0.1 N N_aOH

Solution II : One percent $CuSo_4\,5H_2O$

Solution III : Two percent K Na tartarate

Fifty ml of solution_I was mixed with 0.5 ml of solution and 0.5 ml of solution III.

Folin-Ciocalteu reagent

Solution of tungstate and sodium molybdate in phosphoric acid and hydrochloric acid was purchased from Gisco Research Laboratory Pvt. Ltd. Bombay. The reagent was stored in the refrigerator and protein was diluted with an equal volume of water before use. Five m1 alkaline solution was added to ml of test solution and was mixed thoroughly and incubated at 28±2^{o}C for 10 minutes. After 10 minutes 0.5 ml of diluted Folin- Ciocalteu reagent was added and was mixed immediately. The developing colour was recorded at 750 mm on Systronics – 106 Spectrophotometer. Freshly prepared bovine serum albumin was used as standard and values are expressed as micrograms of protein per ml of medium. The results of protein estimation are expressed as net values i.e. the measured value in the test sample minus the sum of values of keratin and fungal controls. The data in the tables are represented up to first decimal figure, which is the mean of three samples.

CONCENTRATION OF HYDROGEN IONS

The pH of the culture filtrate was measured after every incubation period by using Elico pH meter. The initial pH of medium was adjusted 7.0 before sterilization.

RESULTS AND DISCUSSION

The loss in the keratinic pH substrates, changes and protein released into the medium were measured at different incubation periods. The results of keratin decomposition in mineral medium are presented through Tables 1-4.

***Chrysosporium tropicum* GPCK 511**

The results of static and shaking conditions are depicted in Table 1-2. The net protein of culture filtrates from hair was recorded 18.0 ug/ml in the 5 days. In 10 days the value was increased up to 24.6 µg/ml. In 15 days the value was recorded as 26.3 µg/ml. The net protein level in test sample was found in increasing order after 5 days i.e. 29.0, 37.0 and 43.3 µg/ml. in 5, 10 and 15 days, it was found decreased after 15 days and was recorded 30.3 and 23.3 µg/ml. in 20 and 25 days. A gradual loss in weight of keratinous substrate was recorded at different intervals, In 5, 10, 15, 20 and 25 days the weight loss of hair was 19.5, 35.0, 40.0, 31.5 and 20.5 percent in static condition. The pH of hair test sample was 8.5, 8.9, 8.2, 8.3 and 8.1 on same incubation period in static condition. In shaking condition the protein level of test samples were recorded as 20.3, 33.0, 56.0, 44.6 and 31.0 µg/ml on different incubation period while the net protein released in the Culture filtrate from hair was recorded as 8.0, 20.0, 35.0, 19.6 and 10.4 µg/ml. The weight loss was 20.0 35.5 45.5, 40.0 and 33.5 percent in shaking condition in different days. The pH of test sample was found to be 8.0, 8.0 8.1 7.0 and 8.4 in 5, 10, 15, 20 and 25 days.

***Chrysosporium tropicum* GPCK 512**

The results are summarized in Table 3-4. The net protein released in the culture filtrate in static condition was 1.3 ug/ml. in 5 days. In 10 days 5.0 ug/ml protein 20 and 25 days 83.6. and 62.7 ug/ml protein could be recorded. The protein level of test samples was 9.3 16.0, 66.3, 98.3 and 74.3 ug/ml on different incubation periods. Weight of keratinic substrates was found continuously increasing up to 20 days during the decomposition process. Initial weight loss was 8.3 percent in static condition later on it was recorded as 15.5, 55.0, 65.0 and 60.0 percent at 5, 10, 15, 20 and 25 days. The pH of culture filtrate was 8.5, 9.7, 10.0, 9.5 and 9.1 The maximum pH recorded was 10.0 in 15 days and minimum was 8.5 in 5 days. In shaking condition the protein level of test sample was measured as 22.3, 28.0, 167.3, 156.0 and 133.0 ug/ml in 5, 10, 15, 20, and 25 days. The maximum protein was released in 15 was 13.0 µg/ml. In 15 days protein was increased up to 150.3 µg/ml. The net protein level of the test sample was found decreased after 15 days and it was 137.0 µg/ml and 118.0 µg/ml in 20 and 25 days respectively. The weight loss of keratinic substrate was 16.0, 20.0, 82.0, 79.0 and 75.0 percent on different intervals. The pH of mineral medium was found to be 8.4, 7.0, 9.0, and 8.4 in 5, 10, 15, 20, and 25 days.

An effort was made to find out photolytic activity of two different strains of *C. tropicum* during the course of human hair decomposition in mineral medium in the present study. The literature available on keratin decomposition in vitro by keratinophilic fungi and dermatophytes reveald that all the studies were made in static conditions (Kunert, 1972, 1973, 1981; Evans and Hose, 1875; Safranek and Goos, 1982; Deshmukh and Agarwal, 1982; Kushwaha, 1983; Gupta, 1994). The investigation on keratin decomposition by dermatophytes were made by many previous workers and they were of o9pinion that keratinolysis in fungi is by no means an exclusively proteolytic process. The fungi are capable of denaturing the substrate in advance. Most authors assumed that fungi denature keratin by enzymatic reduction of cystine bonds which from the basis of keratin resistance (Chattaway et. al , 1963; Mercer and Verma, 1963; Weary et al. 1965; Weary and Canby, 1967, 1969). Other authors (Mathison, 1963; Zingler and Bohme, 1969) considered the domination of amino acids and peptides with production of ammonia action leading to a gradual denaturation of keratin by alkaline environment. Kunert (1972, 1973) presented the hypothesis for the denaturation of keratin. In the course of keratinolysis, fungus excretes sulfide which cleaves the disulfide bonds of keratin to cystine and sulfocystine. The substrate, denatured by sulfitolysis is then more easily digestible by fungal proteases.

The protease activity can be assumed as the most selective index of physiological change (Kunert, 1972). The protein level of culture media was noted in increasing order with the proceeding incubation periods in case of *C. tropicum* GPCK 511 and *C. tropicum* GPCK

512. In case of *C. tropicum* GPCK 512 the protein level in test samples and net values were found in an increasing order up to 20 days and then protein level of test samples and net protein values were found decreasing in static condition, but in shaking condition it was found increasing after 10 days and decreased after 15 days. The pattern of available the protein level in the decomposing media was found in close proximity with the findings of Kunert (1973) who studied the keratin decomposition by *M. gypseum* in submerged culture. They found declination protein level after 12 days of incubation. Deshmukh and Agarwal(1982) studied the human hair degrading ability of 5 fungi including *C. indicum* and *C. pannicola* and continued the experiment up to 6 weeks. Safranek and Goos (1982) worked on wool decomposition and measured net protein released by some saprophytic fungi up to 14 days. Kushwaha (1983) studied peacock feather decomposition by several keratinolytic and non-keratinolytic fungi in static culture at 30 days of incubation. He found the lower protein values of the test samples by few fungi including *M. gypseum.*

In the current approach, *M*. *gypseum* showed the least value in the first week, which gradually increased on further incubation. The lower values of the test samples probably indicate utilization of soluble non-keratinous protein derived from the hair (Peyton et. al, 1965; Kushwaha 1983). Native keratin substances are complex substances containing an addition to several types of keratin molecules, various non-keratinous organic components capable of providing substantial nutrition without the occurrence of keratinolysis.

In the shaking condition the weight loss in the test sample was high as compared to static condition. In present study the maximum weight loss recorded in the test sample in which the maximum protein was also released. The maximum loss in the hair keratin was caused by *C. tropicum* GPCK 512 after the 10 days in shaking condition. In general the data on protein released by two test fungi were found correspondent to the result recorded in weight loss of keratinic substrates. The pH of test samples of two strains of *C. tropicum* GPCK 511 and *C. tropicum* GPCK 512 was found shifting towards the alkalinity as also noted by Kunert (1979) and Malviya et. al, (1991). The minimum pH recorded was 7.0 in *C. tropicum* GPCK 511 and *C. tropicum* GPCK 512 in shaking condition and that of maximum pH was 8.9 and 10.0 in both the test fungi in static condition. Mathsion (1963) and Ziegler and Bohme (1969) found the medium alkaline with a pH rise upto 9. These authors assumed that the basis of keratinolysis high deamination activity which cause the keratin gradually denatured.

Two distinct patterns were found in the course of the decomposition of keratin. In one trend the alkalization was continuous as the incubation proceeds (with time factor). In the second trend the initial rise in the pH was followed by declination and further rise in pH values were noted. Similar pattern of pH changes were seen by Evans and Hose (1975). Deshmukh and Agarwal (1982) and Kunert (1981). The later author even found modification of the media and explained the three phases alkalization during active growth, modification during the stationary phase and new alkalization during active autolysis phase. The loss in weight of keratin substances. pH change and protein released into the culture medium provide definite evidence of the proteplytic and keratin decomposing ability of *C. tropicum* GPCK 511 and *C. tropicum* GPCK 512. However, the results showed that more protein released and more weight loss was found in shaking condition in comparison to static condition. From the results discussed so far it is clear that maximum values of protein and weight loss were mostly obtained at 10 to 15 days indicating optimum incubation at this period.

TABLE 1
PROTEIN RELEASED (µG/ML) AND WEIGHT LOSS (%) OF HUMAN HAIR DURING THE GROWTH OF CHRYSOSPORIUM TROPICUM GPCK 511 IN STATIC CONDITIONS.

Incubation Period in Days	Fungus Control	Keratin Control	Test Sample	Sum of keratin and fungus control	Net Protein Released	pH	Weight Loss
5	8.0 ± 1.0	3.0 ± 0.0	29.0 ± 1.0	11.0 ± 1.0	18.0 ± 3.6	8.5	19.5
10	9.0 ± 2.6	3.3 ± 0.0	37.0 ± 6.0	12.3 ± 2.3	24.6 ± 5.1	8.9	35.0
15	13.3 ± 4.1	3.6 ± 0.5	43.3 ± 5.1	17.0 ± 3.6	26.3 ± 7.5	8.2	40.0
20	15.3 ± 4.7	4.3 ± 0.5	30.3 ± 9.5	19.6 ± 4.9	10.7 ± 5.5	8.3	31.5
25	6.6 ± 1.5	4.3 ± 0.5	23.3 ± 4.1	11.0 ± 1. 7	12.3 ± 4.0	8.1	20.5

TABLE 2
PROTEIN RELEASED (µG/ML) AND WEIGHT LOSS (%) OF HUMAN HAIR DURING THE GROWTH OF CHRYSOSPORIUM TROPICUM GPCK 511 IN SHAKING CONDITIONS.

Incubation Period in Days	Fungus Control	Keratin Control	Test Sample	Sum of keratin and fungus control	Net Protein Released	pH	Weight Loss
5	7.0 ± 2.0	5.3 ± 0.5	20.3 ± 0.5	12.3 ± 2.0	8.0 ± 1.5	8.5	20.0
10	7.3 ± 0.5	5.6 ± 0.5	33.0 ± 8.3	13.0 ± 1.0	20.0 ± 7.6	8.0	35.5
15	14.6 ± 3.0	6.3 ± 0.5	56.0 ± 6.0	21.0 ± 2.6	35.0 ± 4.3	8.1	45.5
20	18.3 ± 1.1	6.6 ± 0.5	44.6 ± 7.5	24.9 ± 2.9	19.6 ± 8.7	7.0	40.0
25	14.0 ± 4.5	6.6 ± 0.5	31.0 ± 2.8	20.6 ± 2.0	10.4 ± 4.3	8.4	33.5

TABLE 3
PROTEIN RELEASED (µG/ML) AND WEIGHT LOSS (%) OF HUMAN HAIR DURING THE GROWTH OF CHRYSOSPORIUM TROPICUM GPCK 512 IN STATIC CONDITIONS.

Incubation Period i Days	Fungus Control	Keratin Control	Test Sample	Sum of keratin and fungus control	Net Protein Released	pH	Weight Loss
5	5.0±1.0	3.3 ± 1.1	43.3 ± 5.0	8.3 ± 1.5	35.5 ± 5.5	8.0	25.5
10	7.6±1.1	4.6 ± 0.5	64.3 ±11.5	12.2 ± 1.5	52.1 ± 10.5	9.2	39.0
15	8.6±2.0	5.3 ± 0.5	71. 6 ± 6.1	14.0 ± 1.7	57.6 ± 6.0	8.5	40.5
20	10.0±2.0	5.6 ± 0.5	77.6 ± 5.0	15.6 ± 2.0	62.0 ± 7.3	9.0	46.0
25	7.3± 1.5	5.6 ± 0.5	88.3 ± 3.7	13.0 ± 1.7	75.3 ± 3.0	8.5	64.0

TABLE 4
PROTEIN RELEASED (µG/ML) AND WEIGHT LOSS (%) OF HUMAN HAIR DURING THE GROWTH OF CHRYSOSPORIUM TROPICUM GPCK 512 IN SHAKING CONDITIONS.

Incubation Period in Days	Fungus Control	Keratin Control	Test Sample	Sum of keratin and fungus control	Net Protein Released	pH	Weight Loss
5	8.6±2.10	0.5	5.3 ±1.22	0.5	22.3 ±1.00	3.7	14.0 ±2.00
10	9.3±1.50	0.5	5.6 ±2.00	0.5	28.0 ±2.05	4.3	15.0 ±2.05
15	10.6±3.00	0.5	6.3 ±0.08	0.5	167.3 ±22.50	7.6	17.0 ±1.20
20	12.3±1.08	4.1	6.6 ±0.05	0.5	156.0±13.4	19.0	4.3±1.00
25	7.6 ±2.04	0.5	7.3 ±1.06	0.5	133.0±13.0	15.0	1.0±0.00

ACKNOWLEDGEMENT

We are thankful to The Principal and Head of Botany Department for research facilities.

REFERENCES

1. Chattaway, F.W., Ellis, D.A. and Barlow, A.J.E. 1963. Kertnases of dermatophytes. J. Invest. Dermatol. 41 : 31-37.
2. Chesters, C.G.C. and Mathison, C.E. 1963. The decomposition of wool keratin by keratinomyces ajelloi. Sabouraudia. 2 : 225-237.
3. Deshmukh, S.K. and Agrawal, S.C. 1982. In vitro degradation of hair by some dermatophytes and other keratinophilic fungi. human hair by some keratinophilic fungL Mykosen. 25 : 454-458.
4. Deshmukh. S.K. and Agrawal. S.C. 1985. Degradation of human hair by some dermatophytes and other keratinophilic fungi. Mykosen. 28 : 463-466.
5. De Vries. 1962. Keratinophilic fungi and their action. Antonie Van Leeuwenhock.28 : 121-133.
6. Evans. E.G.V. and Hose. H. 1975. In vitro degradation of human hair by *Hendersonula toruloidea.* Subouraudla. 13 : 323-328.
7. Evolceanu, 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 .
8. Evolceanu, R. Alteras, J. and stoian, M. 1963. Consideration sur la morphologie le pouvoir phathogene et la geophil1e du the *Microsporum nanum*. Mycopath. Mycol. Appl. 19 : 24-36.
9. Gupta. M. 1994. Decomposition of hair and feathers by keratinophilic fungi. Ph.D. Thesis. Kanpur University, Kanpur.
10. Kunert. J. 1973. Keratin decomposition by dermatophytes. J. Zeistchrift fur Allg. Mickrobiologie, 13 : 489-498.
11. Kunert. J. and Krajci, D. 1981. An electron microscopy study of keratin degradation by the fungus *Microsporum gypseum in* vitro. Mykosen. 24 : 485-496.
12. Kunert, J. 1995. Biochemical mechanism of keratin degradation by keratinophilic ftmg1. J. Indian. Bot. Soc. 74 : 89-98.
13. Kushwaha. R.K.S. 1983. The in vitro degradation of peacock feathers by some ftmg1. Mykosen. 26 : 324-326.
14. Mathison, G .H. 1963. The microbial decomposition of keratin. Proceeding of the International Colloquium on Medical Mycology. Antwerp. 6 : 179-203.
15. Mathison. G.E. 1965. An autoradtographic technique for the detection of cystine and pepudes containing cystine or its derivatives in keratin digestion. Mycopathol. Mycol. Appl. 27 : 225-232.
16. Mercer, E.H. 1961. Keratin and keratinization. An Ssa Pesgam on Press Oxford.
17. Mercer. E. H. and Verma. B. S. 1963. Hair digested by *Trichophyton mentagrophytes*. J. Invest. Dermatol. 48 : 357-360.
18. Nickerson. W. J. 1947. Biology of pathogenic fungi. Chronica Botaniea Co. International Science Publishers.
19. Nickerson. W.J. and Durand. S.C. 1963. Keratinase II. properties of crystalline enzyme. Biochem. Biophys. Acta. 77 : 87-99.
20. Peyton, E. Weary. P.E., Canby. C.M. and Cawley. E.P. 1965. Keratinolytic activity of *Microsporum canis* and *Microsporum gypseum*. Jour. Invest. Dermatology. 44 : 300-310.
21. Ragot , Jacqueline. 1966. Acttvite

protiolytique due Keratinomyces Je11 01 vanbr. Comptes Rendus de I, Academine des Science de Paris. Ser. D. 263 : 1895-1898.
22. Ragot , Jacqueline 1967. Activite proteolytique du ICeratinomyces aje1101 vanbr. Cultive en presence de Keratine. Comptes Rendus de l' Academine des Science de Paris, Ser. D. 265 : 493-496.
23. Ragot, Jacqueline. 1968. Comportement. due Keratinomyces aje110i vanbr. In vitro son activita proteolytique at keratinolytique these pour a btenirle grade de docteur, Faculte des Sciences Uni varsity de Toulouse.
24. Safranek. W.W. and Goos , R.D.1982. Degradation of wool by saprophytic fungi. Can. J. Microbial. 28 : 138-140.
25. Singh, G. and Agrawal, S.C. 1987. Role of proteolytic enzymes produced by certain keratinophilic fungi in keratin degradation. Science and Culture. 53 : 353-356.
26. Stahl. W.H. Mcqua , B., Mandel's, G.R. and Siu, R.G.H. 1949. Studies on the microbIological degradation of wool. Sulphur metabolism. Arch. Biochem. 20 : 422-432.
27. Stahl, W .H., Mcque, B., Mandels, G.R. and Siu, R.G.H. 1950. Studies on the microbiological degradation of wool. II Nitrogen Metaboism. Arch. Biochem. 27 : 211-220.
28. Stahl, W. H. Mcque, B. Mendels, G. R. and Siu, R.G. H. 1950. Studies on the microb1.o1ogical degradation of wool III Digestion of norm al and modified brit tlar proteins. Text. Res. J. 20 570-579.
29. Weary, P. E. Canby, C. M. and Cawley, E. P. 1965. Keratinolytlc activity of *Microsporum canis* and *M. gypseum.* J. Invest, Dermatol. 300-310.
30. Weary, P. E. and Canby, C. M. 1967. Keratinolytic activity of *Trichophyton schoenleini, T. rubrum and T. mentagrophytes.* J. Invest: Dermatol. 48 : 240-248.
31. Weary, P.E. and Canby. C.M. 1969. Further observation on the kerattnolytic activity of *Trichophyton schoenleini* and *T. rubrum.* J. Invest. Dermatol. 53 : 58-63.
32. Yu, R.J., Harman. S.R. and Blank, F. 1969. Hair digestion by a keratinase of *Trichophyton mentagrophytes.* J. Invest. Dermatol. 53 : 166-170.
33. Yu. R.J., Harman, S.R. and Blank, F. 1968. Isolation and purification of an extracellular keratlnase of *Trichophyton mentegrophytes. J. Bact. 96 : 1435.*
34. Yu, R.J., Harman. S.R., Grappel, S.F. and Blank, F. 1971. Two cell. bound keratinase of *Trichophyton mentagrophytes*. Jour. Invest. Dermatol. 56 : 27-32.
35. Yu, R.J. Ragot, J. and Blank, F. 1972. Keratinase hydrolysis of keratinous substrates by three enzymes of *Trichophyton mentagrophytes.* Experientia. 28 : 1512-1513.
36. Ziegler, H.1967. Vergleichende Untersuchungen uber den stoffwechesl von Schimmelpilzen and dermatophyten 2 keratinabbau. Z. Allg. Microbial. 7 : 53-75.
37. Ziegler, H. and Reichmann, G. 1968. Uber den Schwefelstoffwechsel von *Microsporum canis*. Mykosen. 11 : 903-907
38. Ziegler, H., Bohme, Hannelore and Reichmann, G. 1969. Stoffwechsal physiologiscne Untersuchungen uber den Abbau Jon Proteinen durch *Microsporum gypseum and Microsporum canis*. Dermatol. Mschr. 155 : 835-856.