Influence of Keratinous Substrates on Keratinolytic Ability of Chrysosporium tropicum GPCK 511 and Chrysosporium tropicum GPCK 512

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Abstract - Keratinolytic activity of C. tropicum GPCK 511 and C. tropicum GPCK 512 has not been yet investigated on other keratin sources such as horse hair, goat, hair, cow hair and fathers. In the present study we, therefore, report the keratinolytic ability of C. tropicum strains on different keratin sources with a view to determine degradation of various keratinous substrates in static and shaking condition as evidenced by protein release and weight loss.

Keywords: Chrysosporium tropicum, keratinolytic ability.

1. INTRODUCTION

Chemically skin, hair nails wool horn and feathers are made up of special type of animal proteins which are fibrous in nature and are characterized by their stability towards water, dilute acids and alkalies. These proteins are derived from ectodermal cells and because of their typical structural feature they are not easily hydrolysed by most of the protelytic enzymes. All such animal proteins when grouped together are termed as keratin. Biologically the keratin offer mechanical protection to the animal body and preserve if from bacterial attack. They are also economically important because wool, feathers and hair of some animals are commercially useful [1]. The stability of keratin has been a subject of interest for the last several years. This complex structure originates through on extreme development of the cytoskeleton formed by intermediate filaments. Therefore structure of keratin mostly corresponds to the filaments and matrix model. These filaments are formed by cytokeratins which are filamentous protein composed of a central a-helical domain and to amorphous end domains. They have a low content of cystine. By self assembly they formed dimmers, tetramers, octamers and finally intermediate filaments from 32 polypeptide chain on average. In keratin filaments are embedded in the matrix formed by filaments associated proteins. The matrix is rich in cystine which formed numerous disulphide bridge both among various matrix protein and also among the matrix proteins and end domains of cytokeratins. These interchanged bridges are main source of the high resistance and insoluble keratins [2].

Various keratinous substrates in the form of hairs, nails, horn, hooves, feathers and wool are abundantly present in nature. The observation that these keratinous substrates are attacked by or infected by specific group of microorganisms has led to tremendous activity in recent years in the biodegradation of keratins which was otherwise very slow by other chemicals and protelytic enzymes. Keratinophilic fungi constitute a group of fungi which when cultured in media containing keratin may degrade and use this compound as a basic source of carbon and nitrogen. However, the way in which these

fungi attack and digest keratin is not well known [3]. Some authors have reported that mycelium of fungi mechanically first penetrates into keratin and then there is a possibility of enzyme release from these fungi resulting into degradation of keratin [4], [5], [6], [7], [8], [9], [10].

However, keratinolytic activity of *C. tropicum* GPCK 511 and *C. tropicum* GPCK 512 has not been yet investigated on other keratin sources such as horse hair, goat, hair, cow hair and fathers. In the present study we, therefore, report the keratinolytic ability of *C. tropicum* strains on different keratin sources with a view to determine degradation of various keratinous substrates.

2. MATERIALS AND METHODS

The following keratin substrates were used to study their decomposition by two different strains of *C. tropicum* i.e. GPCK 511 and GPCK 512. 1. Horse hair 2. Goad hair 3. Cow hair 4. Hen hair.

All keratinous substrates i.e. horse hair, goad hair, cow hair, hen feather were washed with sterilized water, cut into short fragments and weighed into 200 mg aliquots. The basal medium contained the following ingredients per litre of glass distilled water : K_2HPO_4-1.0 gm; $MgSO_4.7H_2O$-O.5 gm KCl-O.5gm; $NaNO_3$-2.0 gm: $FeSO_4.7H_2O$-0.01 gm and sucrose-30 gm.

Two hundred fifty ml Erilenmeyer flasks containing 50 ml of mineral medium and 200 mg of keratin substrates (horse hair, goad hair, cow hair, hen feather) were autoclaved at 15 1bs pressure for 10 minutes. The protein present in the medium was substracted from the controls. The flasks were inoculated with 2 ml spore suspension. The spore suspension was obtained from the surface of 6 days old culture previously grown on mineral medium by brushing spores in 5 ml of sterilized distilled water and 2 ml of this spore suspension was added to each flask. The following control flasks were run.

1. Keratin Control
   (i) To which were added 50 ml of mineral medium and 200mg of horse hair.
   (ii) To which were added 50 ml of mineral medium and 200 mg of goat hair.
   (iii) To which were added 50 ml of mineral medium and 200 mg of cow hair.
   (iv) To which were added 50 ml of mineral medium and 200 mg of hen feather.
2. Fungus control to which were added 50 ml of mineral medium and fungal inoculum.
3. Test sample to which were added 50 ml of mineral medium and 200 mg of keratin substrate separately and fungal inoculum.

The flasks were incubated as static and in shaking condition at the rate of 12 hours daily at 28±2^oC and filtered after 5, 10, 15, 20, and 25 days.

2.1 SUBSTRATE DECOMPOSITION

The decomposition of substrate was assessed by following the method of Garrett [11] and expressed as weight loss. After incubation for desired period, substrates from the test flasks and keratin controls were removed and dried at 80^oC. The filtrates were tested for protein released. The substrates neither washed nor the mycelium was removed. The dry weight of one was of horse hair, cow hair and hen feather at desired incubation period was compared to its initial weight and controls. If the substrate in the test sample minus the fungus control weighed less than at the beginning, the weight loss was calculated and

the average of the weight of the un inoculated keratin substrates was subtracted. If there was still weight loss the fungus was considered keratinolytic and percentage weight loss was calculated. If the substrate plus mycelium weighed more than at the beginning the fungus was considered as non-keratinolytic.

2.2 PROTEIN DETERMINATION

The culture filtrate obtained during different keratinous substrate decomposition, were centrifuged at 4000 rpm for 5 minutes and the supernatant was assyed for protein by using Folin Ciocalteu reagent [12] and [13].. The data in the tables are represented up to first decimal figure, which is the mean of three samples.

2.3 CONCENTRATION OF HYDROGEN IONS

The pH of the culture filtrate was measured after desired days of incubation by pH meter, while the initial pH of mineral medium was 7.0

3. RESULTS AND DISCUSSION

Experiments performed with *C. tropicum* i.e. GPCK 511 on various keratin sources in static and shaking conditions are given in Tables 1-2.

3.1 KERATINOLYTIC ABILITY USING HORSE HAIR

Chrysosporium tropicum GPCK 511

Horse hair was degraded in mineral medium in static condition and 21.0µg/ml protein of test sample was released in 5 days. It showd increasing trend with the increase in incubation period upto 15 days. Protein released in test sample during 10, 15, 20 and 25 days were recorded to be 59.0, 109.0 68.3 and 49.3 µg/ml respectively. The net protein release from the horse hair also showed similar trend recording maximum net protein release (88.4 µg/ml whereas it further decreased to 30.3 µg/ml in 25 days. In the beginning of all the experiment pH was maintained as 7.0. It was also measured at different incubation periods to find any change in the pH values which ranged from 7.0 to 8.0 after the completion of incubation periods. The percentage loss in weight of horse hair in static.condition was also calculated after the incubation period. The percent loss of horse hair varied depending on the rate of loss was found to be from 8.5 to 55.0 percent. In another set of experiment, experimental material was kept on shaker during release in different incubation periods were recorded. The magnitude of protein values of test sample and net protein released in shaking condition was in general more than that of static condition. The values of protein in test sample was maximum during 20 days of incubation, thereafter it decreased during 25 days incubation period. The release of protein in test sample showed linear increase i.e. 31.6, 56.0 and 167.0 µg/ml during 5, 10, 15 and 20 days of incubation period respectively and then declined to 147.3 µg/ml at 25 days. The results of net protein released from the horse hair showed 20.3, 26.0, 37.4, 152.4 and 135.1 µg/ml of protein during 5, 10, 15, 20 and 25 day. The maximum value of net protein released was recorded at 20 days of incubation period thereafter it decreased. As compared to static condition pH values recorded after the end of incubation period showed variation from 8.3 to 9.5 in shaking condition, the maximum pH values were recorded at 25 days of incubation. The increased values in shaking condition might be attributed to better condition of penetration of fungus in keratin substrate and aeraton resulting in superior degradation of keratin. The percentage weight loss in shaking condition also revealed increasing trend during different periods of incubation revealing its maximum value at 20 days of incubation,

thereafter it decreased. From the results obtained it is clear that increase in weight loss is directly proportional to net protein released from the test sample.

Chrysosporium tropicum GPCK 512

In order to determine comparative keratinolytic activity another strain i.e. *C. tropicum* GPCK 512 was also employed under the similar set of reaction conditions. In static condition data on the biodegrading activity of strain GPCK 512 is reported in Table 3 and 4. *C. tropicum* GPCK 512 exhibited maximum protein release at 25 days of incubation *C. tropicum* GPCK 512 showed linear increase up to 25 days of incubation period. The maximum protein values of test sample, net protein and percentage weight loss obtained at 25 days of incubation period were 88.3 µg/ml 75.3 µg/ml and 64.0 per cent respectively. At 5 days incubation lowest values of test sample, net protein and percentage weight loss were recorded. The experiments conducted during shaking condition also indicated wide variations in respect of protein released, net protein and percentages weight loss at Various incubation periods. The results of which are indicated in table 10. The protein released from horse hair in response to keratinolytic action of *C. tropicum* GPCK 512 ranged from 22.3 µg/ml to 167.0µg/ml. maximum protein release of 167.0 µg/ml from test sample was recorded at 15 days of incubation period which then decreased to 156.6 µg/ml and 133.0 µg/ml at 20 and 25 days of incubation. Maximum net protein released from the horse hair was noticed at 15 days of incubation. There after it showed decreasing trend at 20 days. During different incubation periods i.e. 5, 10, 15, 20 and 25 days the differences in net protein were quite larger i.e. 8.1, 12.0, 149.8, 137.6 and 118.7 µg/ml respectively as compared to static condition. At 5 days incubation period pH of the mineral medium was recorded to be 8.0 which remained static after 10 and 15 days of incubation period. After 20 days of incubation it marginally increased to 8.1 and thereafter decreased to 7.9 at 25 days to incubation. Percentage weight loss recorded at 5 days of incubation period was 9.5 which increased to 15.0 per cent after 10 days incubation and then 72.5 per cent 15 days incubation. After wards at 20 days and 25 days incubation it decreased to 70.0 and 64.0 per cent respectively. If the two reaction conditions i.e. static and shaking condition are compared in respect of protein released, net protein and weight loss, it is clear that under static condition there was continuous increase in respect of all these parameters up to 25 days of incubation. However, the keratinolytic action of fungi was about half to that recorded in shaking culture. Under shaking condition the keratinolytic activity was much faster

The two strains of *C. tropicum* used in the experiments also showed differences in respect of their keratinolytic ability to degrade horse hair. For example higher optimum value for protein release at 15 days of incubation period in case of GPCK 511 strain, however, in the experiment with GPCK 512 higher value for protein released was recorded at 25 days of incubation and it was considerable lower than that obtained in the case of *C. tropicum* GPCK 511 under static condition, similarly the net protein released was more with *C. tropicum* GPCK 511. The maximum weight loss (64%) in the case of *C. tropicum* GPCK 512 was recorded at the 25 days. The keratinolytic ability of two strains in shaking condition did not show much differences in respect of protein release, net protein and percentage weight loss, however, keratin degrading ability in terms of protein released and net protein was also relatively faster in case of *C. tropicum* GPCK 511 at 20 days of incubation period as compared to *C. tropicum* GPCK 512

3.2 KERATIONLYTIC ABILITY USING GOAT HAIR

Chrysosporium tropicum GPCK 511

The results of protein released from goat hair and its degradation by *C. tropicum* GPCK 511 were discussed in Table 5. The data on protein released from the test sample showed wide variation at different periods of incubation in static condition. It was noted that protein values of test sample of goat hair also followed similar trend as that of horse hair showing maximum protein released at 20 days of incubation periods. The values of protein released declined considerably above 20 days of incubation period. The values recorded at 5, 10, 15, 20 and 25 days of incubation respect of protein released from test sample were 23.0, 29.0, 62.3, 82.0 and 67.6 µg/ml respectively, similarly net protein release also showed increasing trend from 5 to 20 days of incubation period exhibiting maximum net protein release of 62.4 µg/ml whereas at 25 days of incubation period it was decreased to 49.0 µg/ml. At 5, 10 and 15 days of incubation, the net protein released were 8.4 13.0 and 41.1 µg/ml respectively. The pH values of mineral medium recorded after 5, 10, 15, 20 and 25 days did not show considerable difference and varied from 8.0 to 8.9 The minimum pH value (8.0) and maximum pH value (8.9) was recorded at 10 days of incubation and 5 days incubation periods respectively. Considerable weight loss of keratin substrate in static culture were observed in different incubation periods indicating the impact of incubation period on the biodegradation of *C. tropicum* GPCK 511. The maximum values of 60.0 per cent loss in keratin substrate was recorded at 20 days of incubation which declined to 59.0 per cent at 25 days. The results on protein released, net protein released and weight loss in shaking condition due to growth of *C. tropicum* GPCK 511 in different periods of incubation are shown in Table 6. In shaking condition the rate of keratin degradation of goat hair was considerably faster as evidenced by data on protein released as compare to static condition. Maximum protein released in test sample was recorded at 15 days of incubation (122.3 µg/ml). At 20 days and 25 days of incubation it decreased to 104.0 µg/ml and 53.6 µg/ml. This reflect that up to 15 days incubation period there was rapid growth of fungi due to shaking condition because of the homogeneocity of the media used. At each incubation period values of net protein released were quite high in shaking culture as compare to static culture. The maximum net protein release (98.0 µg/ml) was recorded at 15 days of incubation period. At 25 days of incubation period values of net protein further decreased to (29.6 µg/ml). There was marginal effect of different incubation periods on pH value which varied from 7.1 to 8.4. Percentage weight loss of goat hair in shaking condition is concerned, it was found that maximum values (75.0%) were obtained at 15 days of incubation. It was noted that values for percentage weight loss increased linearly with the increasing incubation period thereafter it decreased at 20 and 25 days.

Chrysosporium tropicum GPCK 512

In order to determine comparative keratinolytic behavior another strain *C. tropicum* GPCK 512 was also grown on goat hair to examine its ability to degrade the same. The results of protein released from goat hair by strain GPCK 51 2 under different incubation periods in static condition is illustrated in Table 7. Results indicate that protein released in the test sample was maximum at 15 days of interval thereafter it decreased. It was noted that minimum value of 90.3 µg/ml in respect of protein released in test sample under static release at this stage was less than that obtained at 5 days of incubation period. The values of 109.3, 119.0 155.6, 114.3 and 90.3 µg/ml in

respect of protein released in test sample were obtained during 5, 10, 15, 20 and 25 days respectively. The net protein content measured during different incubation periods in static condition also revealed similar trend marketing its maximum Value of 139.0 µg/ml at 15 days and thereafter it decreased showing lowest value of 73.0 µg/ml at 25 days of incubation which was lower than the value of 100.0 µg/ml obtained after only 5 days of incubation. It appears that the growth of strain GPCK 512 is faster up to 15 days and hence more degradation of keratin substrate. The lower values of net protein at 20 and 25 days may be attributed to the reutilization of end products. The variation in the pH were from 8.2 to 9.0 during the incubation periods of 5 to 25 days. The weight loss of goat hair recorded was 60.0, 79.5 82.0, 79.0 and 54.0 per cent during incubation periods of 5, 10, 15, 20 and 25 days respectively under static condition. The keratinolytic ability of *C. tropicum* GPCK 512 to degrade goat hair and to release protein under shaking condition was also examined to have an idea about the comparative efficiency of two experimental conditions used (Table 8). Data clearly indicated that maximum value of protein in test sample and net protein content released in the test sample was obtained at 20 days of incubation which decreased thereafter. During the incubation period of 5 to 20 days there was regular increase in net protein released from the test sample. The value (42.6 µg/ml) of protein released in test sample at 5 days of incubation period was recorded which increased to 105.0 at 10 days. At 15, 20 and 25 days of incubation 134.0, 156.3 and 139.6 µg/ml protein was released in the test sample. The net protein was 26.0, 87.0, 113.4, 133.7 and 120.4 µg/ml at 5, 10, 15, 20 and 25 days of intervals respectively. The pH values of culture filtrate obtained after various incubation intervals ranged from 7.6 to 8.1. the incubation periods revealed considerable difference and marked increase up to 20 days and then decreased at 25 days. It was noted that goad hair was degraded up to the extent of 98.0 per cent showing good capability of *C. tropicum* GPCK 512.

If the data of protein released and weight loss of goat hair degradation by two different stains of *C. tropicum* are compared, it becomes evident that keratinolytic activity of *C. tropicum* GPCK 512. The strain GPCK 512 was able to exhibit higher values for protein release, net protein content and percentage weight loss at 15 days of incubation period whereas under the same static conditions strain GPCK 511even after 20 days of incubation period showed almost half keratinolytic activity than the other strain GPCK 512. It could be inferred that under the static condition strain GPCK 512 should be a preferred choice over the other GPCK 511. Under shaking condition *C. tropicum* GPCK 511. Showed optimum values of protein released, net protein and percentage weight loss at 15 days of incubation period. *C. tropicum* GPCK 512 exhibited superior values in respect of protein values of test sample and net protein at both 15 and 20 days of incubation periods. As compared to *C. tropicum* GPCK 511. *C. tropicum* GPCK 512 also showed superior keratinolytic ability by degrading the goat hair to the extent of 98 per cent as compared to 75 percent in the case of *C. tropicum* GPCK 511.

3.3 KERATINOLYTIC ABILITY USING COW HAIR

Chrysosporium tropicum GPCK 511

In our effort to determine keratinolytic ability of *C. tropicum* GPCK 511 and GPCK 512 towards different keratin substrates we have also under taken studies on the degradation of cow hair by above mentioned strains. The result obtained under static condition are reported in Table

9. The samples of cow hair under different incubation periods showed variation from 16.0 to 80.6 µg/ml in respect of protein values obtained from the test sample with

C. tropicum GPCK 511. It was noted that there was constant increase in the protein values with the increasing incubation period showing optimum value (80.6 µg/ml) at 25 days of incubation period. Similar increasing trend was recorded in case of net protein values also showed optimum value of 65.0 µg/ml at 25 days incubation period. The minimum protein release and net protein value recorded at 5 days incubation was 16.0 and 2.4 µg/ml respectively. Variation in the pH of culture filtrate under static condition showed marginal differences of ranging from 7.9 to 8.1. The weight loss of cow hair due to keratinolytic degradation by *C. tropicum* GPCK 511under static condition was recorded to be 7.5, 18.5, 20.0, 50.0 and 56.5 per cent at 5, 10, 15, 20 and 25 days of incubation periods respectively. Under shaking culture (Table 10) strain GPCK 511 performed quite well by degrading cow hair more efficiently as compared to the static condition. The range of variation in respect of protein released was considerably wide from 17.6 to 14.0 µg/ml. Moreover under shaking condition also protein released from the test sample exhibited increasing trend with the increase in days of incubation. The order of protein release recorded during 5, 10, 15, 20 and 25 days was 17.6, 78.6, 101.0, 107.6 and 140.0 µg/ml respectively. It may be noted that net protein values also maintained increasing trend from 5 to 25 days of incubation period. The highest value (116.4 µg/ml) was recorded at 25 days which was followed by 78.6, 78.4, 64.6 and 4.6 µg/ml during 20, 15, 10 and 5 days of incubation. The pH value of the culture filtrate did not exhibit considerable change. The data in Table 10 in respect of weight loss also revealed superior keratinolytic ability of *C. tropicum* GPCK 511 Under shaking condition. The weight loss was recorded in the range of 14.0 to 85.0 per cent during 5 to 25 days of incubation.

Chryssporium tropicum GPCK 512

In order to generate information on the keratinolytic ability of *C. tropicum* GPCK 512 it was inoculated with cow hair in mineral media under static and shaking conditions and then incubated for 5, 10, 15, 20 and 25 days. The results on the protein released and net protein released from the test sample along with percentage weight loss at each incubation periods under static condition is illustrated in Table 11. The results revealed that kerationlytic efficiency of *C. tropicum* GPCK 512 increased linearly up to 20 days of incubation period in respect of net protein released from cow hair. It was interesting to note that during 5 days of incubation period the test sample released 20.6 µg/ml protein which increased to 25.0 and 35.3 µg/ml at 10 and 15 days of incubation respectively. The optimum value of the protein released to the extent of 44.6 µg/ml was observed at 20 days incubation period. Net protein recorded during 5, 10, 15, 20 and 25 days was in the order of 11.0, 10.4, 19.3, 26.6 and 13.7 µg/ml respectively. The effect of incubation periods showed marginal variations in pH values which varied from 8.1 to 8.5 under static condition. The weight loss as influenced by incubation periods showed variations form 15.6 to 39.3 per cent and marked its optimum value at 20 days of incubation periods. Thereafter it came down to 20.5 per cent. The biodegradation of cow hair using *C. tropicum* GPCK 512 was also carried out under shaking condition. The results are given in Table 12. The impact of incubating cow hair with

C. tropicum GPCK 512 for varying periods on the protein released and net protein from the test sample showed

variation. The *C. tropicum* GPCK 512 degraded cow hair to the maximum value at 20 days in respect of protein released under test sample. Incubation beyond 20 days brought considerable decrease in the protein released of the test sample. Similarly net protein released showed linear increase up to 20 days of incubation thereafter it decreased. The maximum values of protein and net protein released from the test sample at 20 days incubation under shaking condition were 107.0 µg/ml and 85.7 µg/ml respectively. The pH scale due to varying incubation periods did not show variations. The weight loss of cow hair due to degradation by *C. tropicum* GPCK 512 under shaking condition ranged from 15.0 to 70.0. The optimum value of weight loss was recorded at 20 days incubation. Considerable difference in the keratinolytic activity of *C. tropicum* GPCK 512 with respect to cow hair were observed under static and shaking condition. The activity of the strain GPCK 512 was observed to be more under shaking condition as compared to static condition. In shaking condition the values of 107.0 µg/ml, 85.7 µg/ml and 70.0 per cent of protein released in test sample, net protein released and weight loss respectively were more than double. Whereas optimum values under static condition were 44.6 µg/ml, 26.6 µg/ml and 39.0 percent for protein, of test sample net protein released and weight loss respectively.

From the results explained so far it is obvious that both the strains i.e. *C. tropicum* GPCK 511 and *C. tropicum* GPCK 512 performed well in respect to degradation of cow hair under of GPCK 511 was more than double in static condition as comparaedd to *C. tropicum* GPCK 512. Under shaking condition the activity of both the strains was superior as compared to respective static condition. Kerationlytic ability using hen feather. In order to determined the effect of keratin substrate on the kerationlytic ability of *C. tropicum* GPCK 511 and *C. tropicum* GPCK 512 it was also considered worthwhile to examine the keratinolysis of hen heather using above two strains under the similar sets of condition.

Chrysosporium tropicum GPCK 511

Under static conditions feather were incubated for varying periods in the presence of *C. tropicum* GPCK 511. It was found that the strain degraded feather releasing varying amount of protein at different incubation periods as shown in Table 13. The maximum value of protein released in the test sample was recorded at 20 days whereas maximum net protein was recorded at 15 days. The protein release in test sample showed increasing trend with increasing incubation period upto 20 days whereas net protein increased to its maximum continuously up to 15 days thereafter it decreased. The maximum values of protein released in test sample and net protein was found to be 52.0 µg/ml and 26.3 µg/ml respectively. Initially at 5 days the protein of test sample and net protein released was very slow in feather. The degree of protein liberation was considerably increased after 10 days of incubation period. There was no appreciable change in the pH value of the mineral media due to varying incubation periods. The variations were from 7.9 to 8.2 only. The weight loss of keratin substrate was determined. The lowest values were recorded at 5 days which increased to 45.5 percent and 50.5 per cent was noticed at 20 days which decreased to 50.0 per cent at 25 days of incubation period. It was noted that the values of weight loss recorded at 15 days and 25 days of incubation were the same. When keratinolytic ability of the C.*tropicum* GPCK 511 towards feather was studied under shaking condition it was found that the strain behaved in a faster way in respect of protein in test sample, net protein. released and percentage weight loss of the test sample at different intervals.

The results are summarized in Table 14. It is evident from the data on protein released from the test sample that it maintained increasing trend up to 20 days thereafter it decreased. It was noted that under shaking culture more than double amount of protein was released at each stage of incubation period. Under shaking condition the rate of protein released at 5, 10, 15, 20 and 25 days recorded was 35.3, 85.0, 100.0, 128.6 and 82.0 µg/ml respectively as compared to 19.6, 39.6, 50.6, 52.0 and 40.0 µg/ml on the same incubation period under static condition. Maximum value of protein released in test sample was recorded at 20 days of incubation period in shaking condition. Similar type of enhancement of net protein release in the test sample was recorded in shaking as well as static condition. The results of net protein released from feather under shaking condition in response to keratinolytic ability of *C. tropicum* GPCK 511 were 20.3, 68.7, 77.7, 110.0 and 65.4 µg/ml at 5, 10, 15, 20 and 25 days respectively, which clearly indicates the superior performance of keratinolytic ability of *C. tropicum* GPCK 511 under shaking condition as campared to static condition. The pH values showed minute variation of 8.9 to 9.2 in the course of incubation during 5 to 25 days. The weight loss ranged from 45 to 82 percent under shaking condition which was superior to that obtained in static condition by the same strain. Maximum value of 82 percent were recorded at 20 days incubation which decreased to 60 percent at 25 days.

Chrysosporium tropicum GPCK 512

The keratinolytic ability of strain GPCK 512 towards feather was also tested under static and shaking cultures. The results obtained in respect of protein in test sample, net protein released, pH value and weight loss under static condition are reported in table 15. It was noted that the strain GPCK 512 was highly active at initial period of incubation i.e. 5 to 10 days releasing 107.5 µg/ml protein at 5 days and 152.3 µg/ml at 10 days incubation. However, its activity was reduced thereafter at 15 days showing 109.6 µg/ml protein release which decreased to 82.o µg/ml at 20 days and was lowest (77.0 µg/ml) at 25 days. Similar activity of the strain GPCK 512 under static condition was noticed at 5 and 10 days indicating 97.2 and 138.3 µg/ml net protein released from the test sample which decreased to 94.3, 64.4 and 62.0 at 15, 20 and 25 days of incubation period. The pH values ranged from 8.4 to 9.8 during 5 to 25 days of experimental periods. The weight loss showed variations from 65.0 to 80 percent recording its maximum value at 10 days incubation. The results on the keratinolytic ability of *C. tropicum* GPCK 512 degrade hen feather under shaking condition are shown in table 22. The range of variation in protein release in test sample, net protein release, pH value and weight loss in shaking conditions were 58.6 to 113.3 µg/ml, 43.6 to 95.3 µg/ml 8.0 to 9.6 and 35.0 to 70.0 percent respectively during 5 to 25 days of incubation. The maximum value for protein released in test sample, net protein, pH value and percentage weight loss were recorded at 15 days incubation period. Thereafter decreasing trend was shown upto at 20 and 25 days of incubation period. *C tropicum* GPCK 512 showed superior keratinolytic activity on hen feather in static condition as compared to shaking condition, moreover the keratinolytic activity of the strain in static condition in respect of protein in test sample, net protein and weight loss was found to be maximum at 10 days incubation period, whereas optimum keratinolytic activity in the shaking condition was of low magnitude even at 15 days incubation period as compared to static condition. The perusal of the data clearly indicate that under static condition keratinolytic ability of the *C. tropicum* GPCK 512 was far superior than the strain GPCK 511 towards

the degradation of hen feather. However, the activity of *C. tropicum* GPCK 511 was superior in shaking condition as compared to *C .tropicum* GPCK 512.

The keratinolytic activity of *C. tropicum* GPCK 511 and *C. tropicum* GPCK 512 strains towards various keratinous sources i.e. horse hair, goat hair, cow hair and hen feather under two sets of reaction condition viz static and shaking cultures revealed differences. The results on the keratinolytic ability of strain GPCK 511 under static condition towards different keratinous sources are compared. Under static condition the keratinolytic ability of *C. tropicum* GPCK 511 was found to be maximum in case of horse hair as compared to goat hair, cow hair and hen feather as this strain released maximum protein of test sample and net protein from the test sample. However, maximum weight loss was recorded in the case of goat hair which was followed by horse hair. Similar trend of the keratinolytic activity of the strain 511 was recorded under shaking condition suggesting that the strain GPCK 511 has more affinity towards horse hair as compared to goat hair, cow hair and hen feather and provide evidence that has maximum ability to degrade and utilize horse hair as source of nutrients. Similar type of work has also been reported on the in vitro degradation of feathers by other fungi [14], [4].

The optimum in vitro keratinolytic activity of *C. tropicum* GPCK 512 static condition was recorded with goat hair as keratin substrate in respect of protein and net protein by the same strain was hen feather whereas under shaking condition the first choice of the strain in degradation and releasing maximum protein of test sample and net protein was favored by strain GPCK 512 and comparatively lower value in respect of protein in test sample and net protein release were recorded. The results are indicating the fact that in addition to the nature of kerationous substrate, the reaction condition also influence the degree of kerationlytic ability of the fungal strains, which varies from strain to strain.

The degree of in vitro degradation and utilization of keratin substrate fungal strains ultimately depends on the structure of keratin which varies in its ability from source to source as the number of disulphide bride present in the keratin structure are variable factor in keratin structure. More the number of disulphide and hence it will be less available for biodegradation by keratinophilic fungus [2], [3], [15], [16]. These findings have also being confirmed by other authors [17], [18]. It has been also reported that the fungus can acquire additional energy by the oxidation of cystine. Ziegler and Recichmann (1968) reports are also available which indicate that peptides containing cystine partially free cystine are formed as an intermediately products in the liquid culture [19], [20], [21]. However, the possibility that the dermatophytes are able to denature keratin by enzymatic reduction of cystine is not well authenticated yet [6].

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TABLE 1 : PROTEIN RELEASED (µg/ml) AND WEIGHT LOSS (%) OF HORSE HAIR DURING THE GROWTH OF *Chrysosporium tropicum* GPCK 511 IN STATIC CONDITION

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	5.6 ± 0.5	21.0 ± 2.6	12.6 ± 2.0	8.4 ± 2.3	8.0	8.5
10	7.3 ± 0.5	6.6 ± 0.5	59.0 ± 6.4	14.0 ± 1.0	45.0 ± 5.5	7.0	25.0
15	14.0 ± 3.0	6.6 ± 0.5	109.0 ± 3.0	12.6 ± 3.5	88.4 ± 5.7	7.3	55.0
20	18.3 ± 1.1	6.6 ± 0.5	68.3 ± 5.5	25.0 ± 1.0	43.3 ± 4.8	7.0	32.5
25	11.6 ± 1.5	7.6 ± 0.5	49.3 ± 9.0	19.0 ± 2.0	30.3 ± 10.3	7.4	25.0

TABLE 2: PROTEIN RELEASED (µg/ml) AND WEIGHT LOSS (%) OF HORSE HAIR DURING THE GROWTH OF *Chrysosporium tropicum* GPCK 511 IN SHAKING CONDITION

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	3.3 ± 1.1	31.6 ± 4.6	11.3 ± 1.5	20.3 ± 5.8	8.3	23.5
10	9.0 ± 2.6	4.6 ± 0.5	39.6 ± 3.0	13.6 ± 2.8	26.0 ± 4.5	8.7	25.0
15	13.3 ± 4.1	5.3 ± 0.5	56.0 ± 12.5	18.6 ± 4.7	37.4 ± 9.8	8.7	39.5
20	9.0 ± 4.9	5.6 ± 0.5	167.0 ± 5.5	14.6 ± 4.3	152.4 ± 9.7	8.5	70.0
25	6.6 ± 1.5	5.6 ± 0.5	147.3 ± 7.0	12.2 ± 1.1	15.1 ± 6.2	9.5	65.0

TABLE 3 : PROTEIN RELEASED (µg/ml) AND WEIGHT LOSS (%) OF HORSE HAIR DURING THE GROWTH OF *Chrysosporium tropicum* GPCK 512 IN STATIC CONDITION

Incubation Period in 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 HORSE HAIR DURING THE GROWTH OF *Chrysosporium tropicum* GPCK 512 IN SHAKING CONDITION

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 ± 0.5	5.6 ± 0.5	22.3 ± 3.7	14.2 ± 0.5	8.1 ± 3.4	8.0	9.5
10	9.3 ± 0.5	6.6 ± 0.5	28.0 ± 4.3	16.0 ± 0.0	12.0 ± 4.3	8.0	15.0
15	10.6 ± 0.5	6.6 ± 0.5	167.0 ± 7.6	17.2 ± 0.5	149.8 ± 7.5	8.0	72.5
20	12.3 ± 4.1	6.6 ± 0.5	156.6 ± 13.4	19.0 ± 3.6	137.6 ± 14.2	8.1	70.0
25	7.6 ± 0.5	6.7 ± 0.5	133.0 ± 13.0	14.3 ± 1.0	118.7 ± 13.5	7.9	64.0

TABLE 5: PROTEIN RELEASED (µg/ml) AND WEIGHT LOSS (%) OF HORSE HAIR DURING THE GROWTH OF *Chrysosporium tropicum* GPCK 511 IN STATIC CONDITION

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	6.6 ± 1.1	23.0 ± 3.5	14.6 ± 0.0	8.4 ± 3.5	8.9	10.5
10	9.0 ± 2.6	7.0 ± 1.0	29.0 ± 1.0	16.0 ± 3.6	13.0 ± 2.6	8.0	15.0
15	13.3 ± 4.1	7.9 ± 0.0	62.3 ± 4.5	21.2 ± 4.1	41.1 ± 8.3	8.5	39.5
20	9.0 ± 4.9	10.6 ± 1.1	82.0 ± 5.2	19.6 ± 5.5	62.4 ± 4.5	8.6	60.0
25	6.6 ± 1.5	12.0 ± 0.0	67.6 ± 10.1	18.6 ± 1.5	49.0 ± 11.2	8.3	59.0

TABLE 6: PROTEIN RELEASED (µg/ml) AND WEIGHT LOSS (%) OF HORSE HAIR DURING THE GROWTH OF *Chrysosporium tropicum* GPCK 511 IN SHAKING CONDITION

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	6.9 ± 0.0	53.0 ± 3.0	14.0 ± 2.0	39.0 ± 4.6	7.1	34.5
10	7.3 ± 0.5	7.1 ± 0.5	102.3 ± 12.5	14.4 ± 1.0	87.9 ± 13.3	8.2	60.0
15	14.0 ± 3.0	10.3 ± 0.5	122.3 ± 3.0	24.3 ± 2.6	98.0 ± 2.5	8.1	75.0
20	18.3 ± 1.1	11.0 ± 0.0	104.0 ± 26.5	29.3 ± 1.1	74.7 ± 25.5	8.0	65.5
25	11.6 ± 1.5	12.3 ± 0.5	53.6 ± 5.6	24.0 ± 1.7	29.6 ± 5.2	8.4	50.0

TABLE 7: PROTEIN RELEASED (µg/ml) AND WEIGHT LOSS (%) OF HORSE HAIR DURING THE GROWTH OF *Chrysosporium tropicum* GPCK 512 IN STATIC CONDITION

Incubation Period in Days	Fungus Control	Keratin Control	Test Sample	Sum of Keratin and Fungus Control	Net Protein Released	pH	Weight Loss
5	5.0 ± 1.0	4.3 ± 0.5	109.3 ± 3.5	9.3 ± 0.5	100.0 ± 3.5	8.2	60.0
10	7.6 ± 1.1	6.0 ± 0.0	119.0 ± 10.5	13.6 ± 0.5	105.4 ± 10.5	8.5	79.5
15	8.6 ± 2.0	8.0 ± 0.0	155.6 ± 19.0	16.6 ± 0.5	139.0 ± 20.3	8.6	82.0

20	10.3 ± 2.0	9.6 ± 0.5	114.3 ± 3.5	20.0 ± 2.6	94.3 ± 2.5	8.9	79.0
25	7.3 ± 1.5	10.0 ± 0.0	90.3 ± 2.0	17.3 ± 1.5	73.0 ± 3.4	9.0	54.0

TABLE 8: PROTEIN RELEASED (µg/ml) AND WEIGHT LOSS (%) OF HORSE HAIR DURING THE GROWTH OF *Chrysosporium tropicum* GPCK 512 IN SHAKING CONDITION

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 ± 0.5	8.0 ± 0.5	42.6 ± 4.6	16.6 ± 0.5	26.0 ± 4.9	7.6	31.5
10	9.3 ± 0.5	8.6 ± 0.5	105.0 ± 9.6	18.0 ± 0.0	87.0 ± 9.6	7.6	54.0
15	10.6 ± 0.5	10.0 ± 0.0	134.0 ± 11.2	20.6 ± 0.5	113.4 ± 11.5	8.1	65.0
20	12.3 ± 0.5	10.3 ± 0.5	156.3 ± 1.5	22.6 ± 4.0	133.7 ± 3.0	8.0	98.0
25	7.6 ± 0.5	11.6 ± 1.5	139.6 ± 1.5	19.2 ± 1.5	120.4 ± 9.8	7.9	72.5

TABLE 9: PROTEIN RELEASED (µg/ml) AND WEIGHT LOSS (%) OF HORSE HAIR DURING THE GROWTH OF *Chrysosporium tropicum* GPCK 511 IN STATIC CONDITION

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	5.6 ± 0.5	16.0 ± 1.7	13.6 ± 0.5	2.4 ± 0.5	7.9	7.5
10	9.0 ± 2.6	6.0 ± 0.0	21.0 ± 1.0	15.0 ± 3.2	6.0 ± 2.5	8.0	18.5
15	13.3 ± 4.1	6.6 ± 0.5	32.3 ± 7.2	20.0 ± 4.3	12.3 ± 6.1	7.9	20.0
20	9.0 ± 4.9	8.3 ± 0.5	62.3 ± 8.5	17.3 ± 5.1	45.0 ± 3.7	8.0	50.0
25	6.6 ± 1.5	9.0 ± 0.0	80.6 ± 6.6	15.6 ± 1.5	65.0 ± 6.0	8.1	56.5

TABLE 10: PROTEIN RELEASED (µg/ml) AND WEIGHT LOSS (%) OF HORSE HAIR DURING THE GROWTH OF *Chrysosporium tropicum* GPCK 511 IN SHAKING CONDITION

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	6.0 ± 0.0	17.6 ± 1.5	13.0 ± 2.0	4.6 ± 3.2	7.9	14.0
10	7.3 ± 0.5	6.6 ± 0.5	78.6 ± 6.1	14.0 ± 1.0	64.6 ± 6.5	7.8	45.0
15	14.0 ± 3.0	8.6 ± 0.5	101.0 ± 2.6	22.6 ± 2.8	78.4 ± 5.0	7.5	65.0
20	18.3 ± 1.1	10.6 ± 0.5	107.6 ± 9.5	28.9 ± 4.0	78.7 ± 13.0	7.0	69.0
25	11.6 ± 1.5	12.0 ± 0.0	140.0 ± 18.5	23.6 ± 1.5	116.4 ± 17.0	7.0	85.0

TABLE 11: PROTEIN RELEASED (µg/ml) AND WEIGHT LOSS (%) OF HORSE HAIR DURING THE GROWTH OF *Chrysosporium tropicum* GPCK 512 IN STATIC CONDITION

Incubation Period in Days	Fungus Control	Keratin Control	Test Sample	Sum of Keratin and Fungus Control	Net Protein Released	pH	Weight Loss
5	5.0 ± 1.0	4.6 ± 0.5	20.6 ± 1.1	9.6 ± 0.5	11.0 ± 1.1	8.1	15.6
10	7.6 ± 1.1	7.0 ± 0.0	25.0 ± 6.2	14.6 ± 1.1	10.4 ± 3.0	8.2	20.0
15	8.6 ± 2.0	7.3 ± 0.5	35.3 ± 3.0	16.0 ± 2.6	19.3 ± 6.0	8.4	25.0
20	10.3 ± 2.0	7.6 ± 0.5	44.6 ± 4.1	18.0 ± 2.0	26.6 ± 4.1	8.5	39.0
25	7.3 ± 1.5	8.0 ± 1.0	29.0 ± 4.1	15.3 ± 2.5	13.7 ± 2.5	8.2	20.5

TABLE 12: PROTEIN RELEASED (µg/ml) AND WEIGHT LOSS (%) OF HORSE HAIR DURING THE GROWTH OF *Chrysosporium tropicum* GPCK 512 IN SHAKING CONDITION

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 ± 0.5	7.6 ± 0.5	18.6 ± 1.1	16.2 ± 1.1	2.4 ± 2.0	8.0	15.0
10	9.3 ± 0.5	7.6 ± 0.5	35.6 ± 1.1	17.0 ± 0.0	18.6 ± 1.1	7.9	28.0
15	10.6 ± 0.5	8.6 ± 0.5	66.0 ± 5.2	19.2 ± 0.5	46.8 ± 4.7	8.0	58.5
20	12.3 ± 4.1	9.0 ± 0.0	107.0 ± 3.0	21.3 ± 4.1	85.7 ± 7.0	8.0	70.0
25	7.6 ± 0.5	9.6 ± 0.5	40.0 ± 4.3	17.2 ± 1.1	22.8 ± 4.0	7.9	35.0

TABLE 13: PROTEIN RELEASED (µg/ml) AND WEIGHT LOSS (%) OF HORSE HAIR DURING THE GROWTH OF *Chrysosporium tropicum* GPCK 511 IN STATIC CONDITION

Incubation Period in Days	Fungus Control	Keratin Control	Test Sample	Sum of Keratin and Fungus Control	Net Protein Released	pH	Weight Loss
5	7.2 ± 2.0	6.6 ± 0.5	19.6 ± 2.1	13.2 ± 2.5	6.4 ± 3.5	8.0	16.8
10	7.3 ± 0.5	6.3 ± 1.1	39.6 ± 2.5	13.6 ± 0.5	26.0 ± 3.0	8.2	45.5
15	14.0 ± 0.5	10.3 ± 0.5	50.6 ± 1.5	24.3 ± 2.6	26.3 ± 2.0	7.9	50.5
20	18.3 ± 1.1	11.6 ± 0.5	52.0 ± 5.8	30.0 ± 1.7	22.0 ± 4.9	8.0	55.0
25	11.6 ± 1.5	12.3 ± 0.5	40.0 ± 1.0	24.0 ± 2.0	16.0 ± 2.6	8.1	50.0

TABLE 14: PROTEIN RELEASED (µg/ml) AND WEIGHT LOSS (%) OF HORSE HAIR DURING THE GROWTH OF *Chrysosporium tropicum* GPCK 511 IN SHAKING CONDITION

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	7.0 ± 0.0	35.3 ± 2.0	15.0 ± 1.0	20.3 ± 1.5	9.0	45.0
10	9.0 ± 2.6	7.3 ± 0.5	85.0 ± 2.6	16.3 ± 2.6	68.7 ± 5.2	8.9	65.0
15	13.3 ± 4.1	9.0 ± 0.0	100.0 ± 8.7	20.3 ± 4.1	77.7 ± 3.0	9.2	70.0
20	9.0 ± 4.9	9.6 ± 0.5	128.6 ± 0.5	18.6 ± 5.2	110.0 ± 4.7	9.0	82.0
25	6.6 ± 1.5	10.0 ± 0.0	82.0 ± 2.6	16.6 ± 1.5	65.4 ± 4.1	9.1	60.0

TABLE 15: PROTEIN RELEASED (µg/ml) AND WEIGHT LOSS (%) OF HORSE HAIR DURING THE GROWTH OF *Chrysosporium tropicum* GPCK 512 IN STATIC CONDITION

Incubation Period in Days	Fungus Control	Keratin Control	Test Sample	Sum of Keratin and Fungus Control	Net Protein Released	pH	Weight Loss
5	5.0 ± 1.0	5.3 ± 0.5	107.5 ± 1.5	10.3 ± 0.5	97.2 ± 1.5	9.0	65.5
10	7.6 ± 1.1	6.3 ± 0.5	152.3 ± 28.5	14.0 ± 1.0	138.3 ± 29.5	9.5	80.0
15	8.6 ± 2.0	6.6 ± 0.5	109.6 ± 15.5	15.3 ± 2.3	94.3 ± 15.6	9.8	65.0
20	10.0 ± 2.0	7.6 ± 0.5	82.0 ± 3.4	17.6 ± 2.6	64.4 ± 3.6	8.4	60.5

25	7.3 ± 1.5	7.6 ± 0.5	77.0 ± 2.6	15.0 ± 1.7	62.0 ± 1.0	8.7	49.0

TABLE 16: PROTEIN RELEASED (µg/ml) AND WEIGHT LOSS (%) OF HORSE HAIR DURING THE GROWTH OF *Chrysosporium tropicum* GPCK 512 IN SHAKING CONDITION

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 ± 0.5	6.3 ± 0.5	58.6 ± 11.9	15.0 ± 1.0	43.6 ± 10.9	8.0	35.0
10	9.3 ± 0.5	7.0 ± 0.0	88.6 ± 18.6	16.3 ± 2.6	72.3 ± 2.6	8.1	65.0
15	10.6 ± 0.5	7.3 ± 0.5	113.3 ± 1.5	18.0 ± 1.0	95.3 ± 1.5	9.6	70.0
20	12.3 ± 4.1	8.6 ± 0.5	93.3 ± 6.6	21.0 ± 4.3	72.3 ± 2.6	8.5	69.0
25	7.6 ± 0.5	9.3 ± 0.5	87.3 ± 9.0	17.0 ± 0.0	70.3 ± 9.0	9.5	63.5