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Influence of moisture content on keratinolytic ability under the Effect of potassium chloride as controlling solute

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Abstract
The effect of water activity controlled through the addition of different concentrations of potassium chloride (aw 0.98 to 0.85) on the keratinolytic activity of two selected the strains of C. tropicum was measured during 5 to 25 days of incubation periods. The results of different water gradient under the Effect of potassium chloride as controlling solute in static condition are then observed .
Keywords: keratinophyllic fungi, water stress, fungal growth

I. Introduction

Water is the medium in which and through which all of the intra and extra cellular chemical reactions and solute transfers, necessary for life, take place. In addition, fungi require substantial amounts of water for the volume increase, responsible for the extension of growth, for optimum growth and to all stages of fungal reproduction (1). However, species differ both in their requirements for water at different stages of the process and in their tolerance of dry conditions. Researchers (2,3) has noted that fungi in soil, unlike plants do not compete for water as their water requirements are so low relative to that present in their immediate environment. However, where fungi are intimately associated with plants they will have to compete with the plant cells for water (3-12). Inspite of, these conventional requirements for water some fungal communities inhabit niches with high osmotic or matric stress or with widely fluctuating water regimes and in such conditions it is likely that fungi do compete for water. Some fungi modify their niche in si a way, as to change its water potential or retentivity others are adapted to germinate, grow and /or suitable condition of low water potential.

II. Objective Of Study

It was therefore, considered worthwhile to determine the keratinolytic ability of C. tropicum GPCK 511 and C. tropicum GPCK 512 under static and shaking condition at different moisture levels to examine the effect of water activity on protein and net protein released in the test sample and percentage weight loss due to biodegradation of human hair as keratin substrate.

III. Material Method

The two strains of C. tropicum were taken for the present study using the following medium. K_2HPO_4-1.0 gm; $MgSO_4.7H_2O$-0.5 gm; KC1-0.5 gm; $NaNO_3$-2.0 gm; $FeSO_4.7H_20$-0.01 gm and sucrose 30 gm per liter of glass distilled water.

Two hundred fifty ml Erilenmeyer flasks containing 50 ml basal medium with desired water activities controlled by KC1, NaCl, sucrose and 200 mg of keratin substrate (human hair) were autoclaved at 15 lbs pressure for 10 minutes. The protein present in the medium was substracted from the controls. The flask 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 added to each flask. The following control flasks were run :

1. Keratin control to which were added 50 ml of desired basal medium and 200 mg of human hair.
2. Fungus control to which were added 50 ml of desired medium and fungal inoculum.
3. Test sample to which were added 50 ml of desired basal medium 200 mg of human hair and fungal inoculum.

The flasks were incubated in static and shaking condition at 28±2°C and filtered after 5, 10, 15, 20 and 25 days for protein released was given in earlier chapter. All the observations were recorded in triplicates. The data in the tables are represented upto first decimal figure which is the mean of three samples.

THE CONCENTRATION OF HYDROGEN IONS

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

IV. Results And Discussion

The effect of water activity controlled through the addition of different concentrations of potassium chloride (aw 0.98 to 0.85) on the keratinolytic activity of both the strains was measured during 5 to 25 days of incubation periods. The results of water activity 0.98 in static condition are reported in Table 1.

TABLE 1:Keratinolytic Ability Of Two Different Strains Of Chrysosporium Tropicum At 0.98 aw Maintained By Using KCl In Static Condition

Incubation Period in Days	Fungus Control (ug/ml)	Keratin Control (ug/ml)	Test Sample (ug/ml)	Sum of Keratin and fungus Control (ug/ml)	Net Protein Released (ug/ml)	PH	Weight Loss (%)
CHRYSOSPORIUM TROPICUM GPCK 511							
5	6.0± 0.0	5.0 ±0.0	16.0 ±0.0	11.0 ±0.0	5.0±0.0	7.9	12.0
10	6.5 ± 0.7	7.0 ±0.0	18.5± 0.0	13.5 ±0.7	5.0 ±0.7	7.9	13.0
15	17.0± 1.4	7.0± 0.0	29.5± 0.7	24.0±1.4	5.5 ±3.5	8.0	18.0
20	22.0± 2.8	7.5 ± 0.7	39.0± 0.0	29.5± 2.8	9.5±2.8	7.0	20.5
25	34.5± 6.3	8.5 ± 0.7	47.5 ±3.5	43.0± 1.4	4.5 ±3.5	7.0	25.0
CHRYSOSPORIUM TROPICUM GPCK 512							
5	8.5 ±0.7	5.0 ±0.0	20.0± 0.0	13.5±0.7	6.5± 0.7	7.5	18.0
10	27.0± 3.5	7.0± 0.0	59.0± 1.4	34.0 ±3.5	34.0 ±3.5	7.3	40.5
15	31.0 ±0.0	7.0± 0.0	61.0 ±1.4	38.0 ±0.0	23.0± 1.4	7.2	45.0
20	117.0 ±2.8	7.5 ± 0.7	171.0 ± 0.7	124.5 ± 3.5	46.5 ± 2.8	7.1	65.0
25	100.0± 0.7	8.5± 0.7	162.0± 3.5	108.5 ±0.0	53.5± 3.5	7.8	50.0

TABLE 2: Keratinolytic Ability Of Two Different Of Strains Chrysosporium Tropicum At 0.98 Maintained By Using KCl In Shaking Condition

Incubation Period in Days	Fungus Control (ug/ml)	Keratin Control (ug/ml)	Test Sample (ug/ml)	Sum of Keratin and fungus Control (ug/ml)	Net Protein Released (ug/ml)	PH	Weight Loss (%)
CHRYSOSPORIUM TROPICUM GPCK 511							
5	12.0 ±0.0	7.0 ±0.0	21.0 ±1.4	19.0± 0.0	2.0 ±1.4	7.5	18.0
10	20.0 ±1.4	8.0±0.0	136.0 ±5.6	28.0 ±1.4	108.0±7.0	7.1	58.0
15	72.5 ±0.7	9.0 ±0.0	149.5 ±7.7	81.5 ±0.7	68.0 ±8.4	7.3	60.0
20	135.5± 4.9	9.5 ±0.7	171.0± 7.0	145.0 ±5.6	26.0 ±12.7	7.2	66.5
25	102.5 ±3.5	11.5± 0.0	131.5 ± 0.7	114.0 ± 3.5	17.5± 2.8	7.2	59.0
CHRYSOSPORIUM TROPICUM GPCK 512							
5	20.0 ±0.0	7.0 ±0.0	38.0 ±2.8	27.0 ±0.0	11.0±2.8	7.2	25.0
10	59.0±9.1	8.0±0.0	79.0 ±1.4	67.0±9.1	12.0 ±10.0	7.3	50.5
15	119.0± 1.4	9.0 ±0.0	168.0 ±1.4	128.0 ± 0.0	40.0± 1.4	7.9	60.0
20	132.0 ±1.4	9.5 ±0.7	180.0 ±0.0	141.5 ± 0.7	38.5 ±7.0	7.3	69.5
25	100.0± 0.0	11.0 ±0.0	171.0 ± 0.0	111.0 ±0.0	60.0 ±0.0	7.2	65.0

At 0.98 aw the net protein released in the culture filtrate from hair was 5.0, 5.0, 5.5, 9.5 and 4.5 ug/ml in 5, 10, 15, 20 and 25 days respectively. The protein released in the test sample was 16.0, 18.5, 29.5, 39.0 and 47.5 ug/ml at 5 to 25 days respectively in the case of C. tropicum GPCK 511. It was noted that values of protein released in test sample increased with increase in period of incubation, however, the net protein showed decreasing trend after 20 days of incubation. Whereas C. tropicum GPCK 512 showed increase in protein release in test sample up to 20 days incubation period. The protein released in test sample and net protein released was 20.0, 59.0, 61.0, 171.0 and 162.0 ug/ml and 6.5, 25.0, 23.0, 46.5 and 53.5 ug/ml in 5, 10, 15, 20 and 25 days respectively in case of C. tropicum GPCK 512. In C. tropicum GPCK 511 the pH of mineral medium was 7.9, 7.9, 8.0, 7.0 and 7.0 at the different incubation period while in C. tropicum GPCK 512 the pH was 7.5, 7.3, 7.2, 7.1 and 7.8 in 5, 10, 15, 20 and 25 days of incubation periods. The maximum weight loss was recorded as 25.0 per cent in 25 days in case of C. tropicum GPCK 511. While it was 65.0 per cent in 20 days in case of C. tropicum GPCK 512.

Chrysosporium tropicum GPCK 511 Chrysosporium Tropicum GPCK 512

A-KCl D-KCl

B - NaCI E - NaCI

C-SUCROSE F-SUCROSE

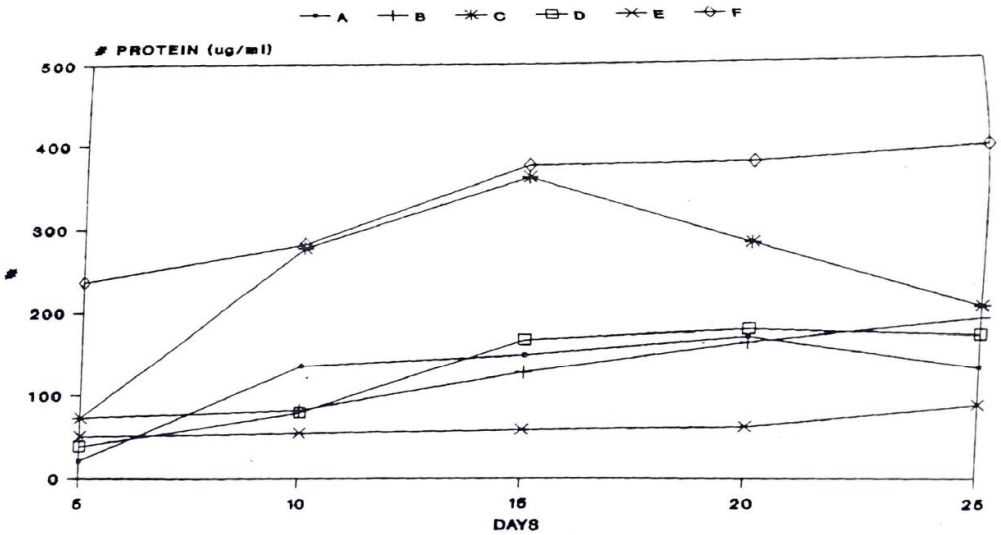

Figure 1 : Protein Released In The Culture Filtrate Of Chrysosporium Tropicum Gpck 511 And Chrysosporium Tropicum Gpck 512 At 0.98 Aw Controlled By KCl, NaCl AND sucrose in shaking condition using human hair.

The effect of water activity (0.98) under shaking condition is given in Table 2 and Fig. 1. It was observed that under shaking condition protein released in test sample increased to 20 days of incubation thereafter decreased at 25 days in the case of GPCK 511 strain. Similar trend was recorded in the case of GPCK 512 upto 20 days of incubation thereafter it decreased. However, net protein released from the test sample exhibited its maximum value at 10 days of incubation thereafter it showed gradual decrease in the case of C. tropicum GPCK 511. Whereas C. tropicum GPCK 512 showed continuous increase in net protein release upto 25 days at the a of 0.98. Variations in pH due to incubation period at this water activity were from 7.1 to 7.3 and 7.2 to 7.9 in case of C. tropicum GPCK 511 and C. tropicum GPCK 512 respectively. Weight loss of the keratin substrate showed maximum value of 66.0 per cent at 20 days in case of C. tropicum GPCK 511. Whereas C. tropicum GPCK 512 showed the maximum values of 69.5 per cent of weight loss (Fig. 1).

Values for protein released in test sample and net protein released obtained under static and shaking conditions indicated the superior ability of both the strains. Weight loss was also considerably higher under shaking condition than static condition.

Chrysosporium Tropicum GPCK 511	Chrysosporium Tropicum GPCK 512
A-KCl	D-KCl
B - NaCI	E - NaCI
C-SUCROSE	F-SUCROSE

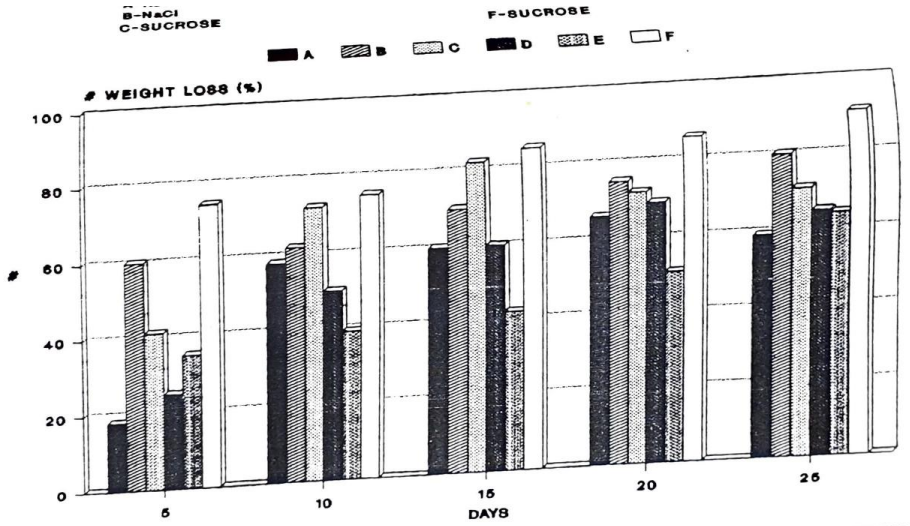

FIG. 2. Weight Loss Human Hair As Indused By Chrysosporium Tropicum Gpck 511 And Chrysosporium Tropicum GPCK 512 AT 0.98 aw Controlled BY KCl, NaCl AND Sucrose In Shaking Condition.

TABLE 3 : Keratinolytic Ability Of Two Different Strains Of Chrysosporium Tropicum At 0.95 aw Maintained By Using KCl In Static Condition.

Incubation Period in Days	Fungus Control (ug/ml)	Keratin Control (ug/ml)	Test Sample (ug/ml)	Sum of Keratin and fungus Control (ug/ml)	Net Protein Released (ug/ml)	PH	Weight Loss (%)
CHRYSOSPORIUM TROPICUM GPCK 511							
5	53.5± 4.9	8.0 ±0.0	82.0± 0.4	61.5 ± 4.9	20.5 ±3.5	7.9	49.0
10	72.5 ±3.5	12.5± 0.7	147.5± 0.7	85.0±4.2	62.5 ±4.9	7.8	60.0
15	110.0 ±0.0	15.0± 0.0	131.0± 1.4	125.0± 0.0	6.0 ±1.4	7.9	58.0
20	65.0 ± 7.0	20.0 ±0.0	86.5±2.1	85.0±0.0	1.5±2.1	6.0	49.5
25	56.0±4.2	20.0 ±0.0	76.5±2.1	76.0±4.2	0.5 ±0.7	7.3	45.0
CHRYSOSPORIUM TROPICUM GPCK 512							
5	42.5± 3.5	8.0 ±0.0	65.5 ± 5.6	50.5 ±3.5	15.0 ±0.7	7.9	40.0
10	50.5± 0.7	12.5 ±0.7	133.0±4.2	63.0 ±0.0	70.0±7.0	5.0	58.5
15	114.5 ±6.3	15.0± 0.0	154.0± 5.6	129.5 ± 6.3	24.5± 1.4	7.5	70.0
20	42.5±2.1	20.0± 0.0	144.0± 1.4	62.5 ± 3.5	81.5 ±4.9	5.0	60.5
25	37.5± 2.1	20.0 ±0.0	120.0 ±0.0	57.5± 2.1	62.5 ±12.1	8.0	50.0

The effect of increasing concentration of KC1 at 0.95 aW did not show considerable influence on the keratinolytic ability of both the strains (Tables 1-2 and Fig. 2). Protein released in the test sample in case of C. tropicum GPCK 511 varied from 76.5 to 147.5 ug/ml recording maximum protein release at 10 days of incubation under static condition whereas net protein released (62.5 ug/ml) showed its maximum value at 10 days incubation thereafter it abruptly declined to 0.5 ug/ml only at 25 days incubation. During various incubation periods weight loss recorded maximum (60.0%) at 10 days of incubation and then decreased to 45.0 per cent at 25 days. Under the similar set of conditions when the strain GPCK 512 was allowed to degrade human hair, it was found that maximum values for protein release in test sample was 154.0 ug/ml at 15 days, net protein release was 81.5 ug/ml at 20 days incubation and weight loss was 70.0 per cent at 15 days of incubation period. All the values recorded in case of GPCK 512 were relatively superior than that of C. tropicum GPCK 511.

In shaking conditions at the same aW both strains performed in a superior way in respect of protein released in test sample net protein and weight loss as shown in Table 3 and Fig. 3. The maximum value for protein release in test sample was 182.0 ug/ml at 20 days in C. tropicum GPCK 511 and 174.5 ug/ml at 15 days in case of C. tropicum GPCK 512. Net protein release was maximum at 25 days in both the strain. The weight loss was shown in Table 4 and Fig. 4. Maximum weight loss at 20 days in case of GPCK 511 recorded was 79.0 per cent whereas it was 74.0 per cent at 15 days in case of GPCK 512. The keratinolytic activity of both the strains was found to be comparatively superior under shaking condition than static conditions at the 0.95 aW when KC1 was used.

TABLE 4: Keratinolytic Ability of two different strains of Chrysosporium Tropicum at 0.95aw maintained by using KCl In Shaking Condition

Incubation Period in Days	Fungus Control (ug/ml)	Keratin Control (ug/ml)	Test Sample (ug/ml)	Sum of Keratin and fungus Control (ug/ml)	Net Protein Released (ug/ml)	PH	Weight Loss (%)
CHRYSOSPORIUM TROPICUM GPCK 511							
5	61.0 ±1.4	8.0 ±0.0	84.0 ±5.6	69.0 ±1.4	15.0±9.1	6.0	50.0
10	114.5 ± 2.1	13.0 ± 0.0	136.0±4.2	127.5± 2.1	8.5 ± 2.1	6.0	65.0
15	119.5 ±0.7	15.5 ±0.7	161.5 ± 7.7	135.0± 1.4	26.5 ±14.3	6.9	72.0
20	123.0 ± 2.8	22.0 ± 0.0	182.0 ± 0.0	145.0 ± 2.8	37.0 ± 2.8	6.8	79.5
25	119.0 ± 1.4	22.5 ± 0.7	181.5 ± 1.4	141.5 ± 0.7	40.0 ± 1.4	6.8	75.0
CHRYSOSPORIUM TROPICUM GPCK 512							
5	45.0 ± 0.0	8.0 ± 0.0	54.0 ± 0.0	53.0 ± 0.0	1.0 ± 0.0	5.0	39.0
10	99.0 ± 1.4	13.0 ± 0.0	142.5 ± 0.7	112.0 ± 1.4	30.5 ± 0.7	5.0	69.5
15	115.0 ± 9.8	15.5 ± 0.7	174.5 ± 6.3	130.5 ± 9.1	44.0 ± 2.1	6.0	74.0
20	92.5 ± 3.5	22.0 ± 0.0	162.5 ± 3.5	114.5 ± 3.5	48.0 ± 4.9	6.1	72.5
25	81.5 ± 2.1	22.5 ± 0.7	160.5 ± 0.7	104.0 ± 2.1	56.5 ± 2.8	6.0	69.0

Chrysosporium tropicum GPCK 611
A-KCI
B-N&CI
C-SUCROSE
Chrysosporium troploum GPCK 612
D-KCI
E-NaCl
F-SUCROSE

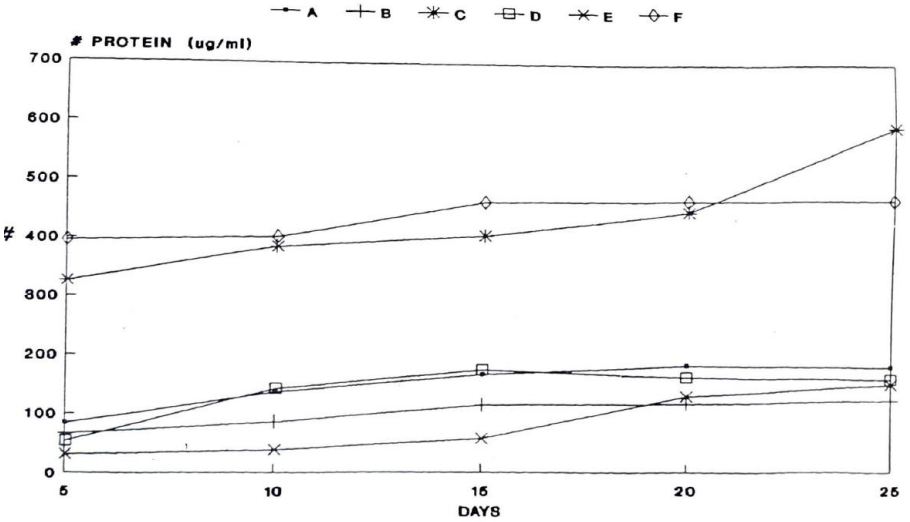

FIG. 3. Protein Released In The Culture Filtrate Of Chrysosporium Tropicum GPCK 511 And Chrysosporium Tropicum GPCK 512 At 0.95 aW Controlled By KCl, NaCl AND Sucrose In Shaking Condition Using Human Hair.

Chrysosporium tropicum GPCK 611
A-KCI
B-N&CI
C-SUCROSE
Chrysosporium troploum GPCK 612
D-KCI
E-NaCl
F-SUCROSE

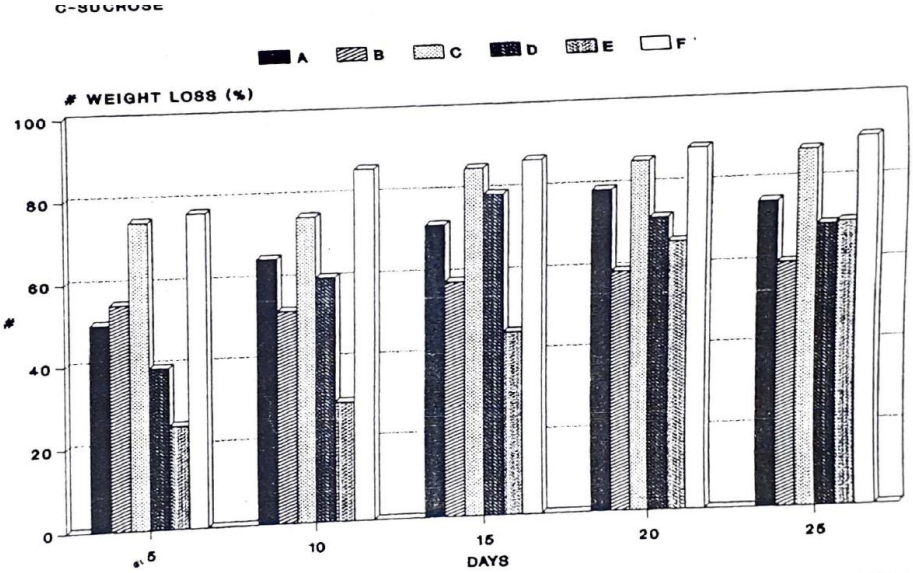

FIG. 4. Weight Loss Of Human Hair As Indused By Chrysosporium Tropicum GPCK 511 And Chrysosporium Tropicum GPCK 512 AT 0.95 aW Controlled by KCl, NaCl AND sucrose in shaking condition.

Results obtained at 0.93 aW are recorded in Tables 3-4 and Fig. 4. sample increase to its maximum value of 145.0 ug/ml at 15 days incubation period in the case of C. tropicum GPCK 511. At 20 days of incubation period it decreased to 134.5 ug/ml and than to 111.0 ug/ml at 25 days of incubation in the static condition. Maximum values of 61.0 ug/ml were recorded in 10 days incubation period whereas maximum weight loss in the case of C. tropicum GPCK 511 was found to be 53.0 per cent at 20 days under static

condition. The response of C. tropicum GPCK 512 under static condition at the a 0.93 (KC1) was somewhat inferior to C. tropicum GPCK 511 in respect of protein of test sample, net protein release and weight loss, as indicated by maximum values of protein released in test sample (120.0 ug/ml

TABLE 5 : Keratinolytic Ability Of Two Different Strains Of Chrysosporium Tropicum AT 0.93 aW Maintained By Using KCl In Static Condition

Incubation Period in Days	Fungus Control (ug/ml)	Keratin Control (ug/ml)	Test Sample (ug/ml)	Sum of Keratin and fungus Control (ug/ml)	Net Protein Released (ug/ml)	PH	Weight Loss (%)
CHRYSOSPORIUM TROPICUM GPCK 511							
5	24.0±1.4	13.5± 0.7	44.0± 1.4	37.5± 0.7	6.5 ±0.7	8.0	24.0
10	46.0 ±2.8	15.0±4.2	122.0 ± 2.8	61.0 ±1.4	61.0± 1.4	5.5	45.0
15	94.5±3.5	20.5± 0.7	145.0 ±1.4	115.0 ±2.8	30.0 ±1.4	5.0	52.0
20	98.5±0.7	25.0±0.0	134.5 ± 6.3	123.5 ± 0.7	11.0±7.0	6.0	53.0
25	62.5±3.5	40.5 ±0.7	111.0 ±1.4	103.0±4.2	8.0 ±2.8	6.0	49.0
CHRYSOSPORIUM TROPICUM GPCK 512							
5	20.5± 0.7	13.5 ± 0.7	50.5± 0.7	34.0±1.7	16.5 ±0.7	6.0	30.0
10	51.0 ±1.4	15.0±4.2	72.5 ±3.5	66.0 ± 5.6	6.5±9.1	7.6	49.0
15	57.0 ±1.4	20.5 ±0.7	110.5 ±2.1	77.5±2.1	33.0±0.0	7.3	50.5
20	70.5± 0.7	25.0± 0.0	120.0± 0.0	95.5± 0.7	24.5± 0.7	7.0	52.2
25	55.0±7.0	40.5 ± 0.7	100.0± 0.0	95.5± 6.3	4.5 ±6.3	7.0	50.0

The effect of a W 0.93 (KCl) in shaking condition appeared to be helpful in increasing the degradation of human hair in respect of protein of test sample, net protein release and weight loss. The protein release from the test sample touched the mark of 182.0 ug/ml 20 days in C. tropicum GPCK 511. However, there was no considerable improvement in net protein release from 5 days to 15 days incubation period. Moreover maximum weight loss (70.0%) was recorded to be at 20 days incubation period (Fig. 4).

Pattern of protein release in test sample indicated maximum value at 15 days thereafter it decrease in the case of C. tropicum GPCK 512 under shaking condition. Maximum value of net protein and percentage weight loss was recorded at 15 days of incubation period. It was 28.0 ug/ml and 60.0. per cent at 15 days respectively.

The effect of 0.90 aw in static condition on hair degradation by both the strains of C. tropicum are given in Table 4. In case of C. tropicum GPCK 511, the net protein released was 18.5, 9.0, 23.0, 35.5 and 11.5 ug/ml in 5, 10, 15, 20 and 25 days respectively. The values of protein release in test sample were 63.0, 65.0, 86.5, 121.0 and 87.0 ug/ml on the same incubation period. The net protein values as well as values in test sample were decreased at 25 days of incubation. The maximum weight loss was 70.5 per cent at 20 days in C. tropicum GPCK 511. At 25, 15, 10 and 5 days it was 62.0, 61.0, 50.5 and 50.0 respectively.

TABLE 6: Keratinolytic Ability Of Two Different Strains Of Chrysosporium Tropicum At 0.93aw Maintained By Using KC1 In Shaking Condition

Incubation Period in Days	Fungus Control (ug/ml)	Keratin Control (ug/ml)	Test Sample (ug/ml)	Sum of Keratin and fungus Control (ug/ml)	Net Protein Released (ug/ml)	PH	Weight Loss (%)
CHRYSOSPORIUM TROPICUM GPCK 511							
5	100.5 ±0.7	14.0±0.0	142.0 ±2.1	114.5 ± 0.7	27.5 ±2.8	6.8	52.0
10	116.5 ± 2.1	17.5±0.7	151.0 ±1.4	134.0± 2.8	17.0±4.2	6.2	60.0
15	124.5 ±6.3	21.0 ±0.0	165.5 ± 4.9	145.5 ± 5.6	20.0 ±10.6	5.0	68.3
20	142.5 ±3.5	32.0±0.0	182.0 ± 1.4	174.5 ±3.5	7.5±4.9	6.3	70.0
25	106.0± 4.9	32.5±0.7	166.0 ± 1.4	138.5 ± 4.2	27.5 ±2.8	6.1	68.5
CHRYSOSPORIUM TROPICUM GPCK 512							
5	81.5 ±16.2	14.0± 0.0	122.5 ± 2.1	95.5 ±16.2	27.0± 18.0	5.1	50.0
10	100.5± 0.0	17.5 ±0.7	131.0 ± 1.4	118.0± 7.0	13.0 ±5.6	5.2	55.0
15	103.5 ± 2.1	21.0 ±0.0	152.5 ± 3.5	124.5± 2.1	28.0± 1.4	5.0	60.0
20	71.0 ±1.4	32.0± 0.0	113.5 ± 0.7	103.0± 1.4	10.5±2.1	5.3	52.0
25	51.0± 1.4	32.5 ±0.7	100.0± 0.0	83.5 ± 0.7	16.5± 2.8	5.4	50.0

TABLE 7: Keratinolytic Ability Of Two Different Strains Of Chrysosporium Tropicum At 0.90 aW Maintained By Using KCl In Static Condition

Incubation Period in Days	Fungus Control (ug/ml)	Keratin Control (ug/ml)	Test Sample (ug/ml)	Sum of Keratin and fungus Control (ug/ml)	Net Protein Released (ug/ml)	PH	Weight Loss (%)
CHRYSOSPORIUM TROPICUM GPCK 511							
5	30.5 ± 0.7	14.0± 0.0	63.0± 2.8	44.5 ±0.7	18.5± 2.1	6.2	50.0
10	40.0± 1.4	16.0± 0.0	65.0 ±0.0	56.0± 1.4	9.0 ±1.4	6.2	50.5
15	47.0 ±2.8	16.5 ±0.7	86.5± 0.7	63.5 ±3.5	23.0 ±2.8	6.1	61.0
20	67.5 ±0.7	18.0 ±0.0	121.0± 2.8	85.5 ±0.7	35.5± 3.5	6.3	70.5
25	55.5 ±4.9	20.0 ± 0.0	87.0 ±2.8	75.5± 4.9	11.5± 7.7	6.4	62.0
CHRYSOSPORIUM TROPICUM GPCK 512							
5	75.5 ±3.5	14.0 ±0.0	100.0± 0.0	89.5 ±3.5	10.5 ±0.7	6.0	65.0
10	80.0±0.0	16.0 ±0.0	108.5± 2.1	96.0 ±0.0	12.5± 5.6	6.1	68.0
15	67.5 ±2.1	16.5 ±0.7	110.0 ±2.8	84.0± 2.8	26.0 ±5.6	5.5	69.5
20	55.0± 1.4	18.0 ±0.0	106.0 ±1.4	73.0 ±1.4	33.0± 0.0	5.0	65.0
25	32.5 ±3.5	20.0± 0.0	93.0± 2.1	52.5 ±3.5	40.5 ±2.8	7.0	64.0

C. tropicum GPCK 512 released more protein in the medium as compared to C. tropicum GPCK 511 in static condition. The protein released in test sample was found to be 100.0, 108.5, 110.0, 106.0 and 93.0 ug/ml at 5, 10, 15, 20 and 25 days respectively. The net protein values were increased upto 25 days. The percentage weight loss of keratin substrate was 65.0, 69.5, 65.0 and 64.0 68.0. per cent at different incubation period. In the case of C. tropicum GPCK 511 the pH of medium controlled by KC1 at 0.90 aW was found 6.2, 6.2, 6.1, 6.3 and 6.4 in 5, 10, 15, 20 and 25 days respectively while in case of C. tropicum GPCK 512 it was varied from 5.0 to 7.0.

The results of protein released, net protein released and percentage weight loss at a 0.90 in shaking condition of (both) the strains are shown in Table 7 and Figs. 3-4. Under shaking condition the maximum protein released in test sample was 145.0 ug/ml in 25 days. In subsequent days it was 75.0, 104.5, 112.0 and 122.5 ug/ml at 5, 10, 15 and 20 days in C. tropicum GPCK 511. However, the net protein release at different incubation periods was found to be -2.5, -17.5, -24.5, 3.5 and 35.0 ug/ml. The negative values shows the sum of keratin and fungus control were more than that of test sample. It was noted that the values of protein in test sample and net protein released were maximum at 25 days in the case of C. tropicum GPCK 511. The pH of medium was 5.2, 5.0, 7.0, 6.7 and 5.1 at 5. 10, 15, 20 and 25 days respectively. The percentage weight loss recorded was 78.0 per cent at 25 days. At 5, 10, 15 and 20 days it was 55.0, 65.0, 70.0 and 73.5 per cent respectively.)

Under the similar set of condition when C. tropicum GPCK 512 allowed to degrade human hair, the maximum value of protein in test sample was 154.5 ug/ml at 25 days, net protein release was 42.0 ug/ml at 25 days. The pH varied from 5.0 to 5.4. The weight loss recorded was 72.0, 75.2, 76.0, 80.0 and 86.0 per cent at 5, 10, 15, 20 and 25 days respectively. It was noticed that all the values recorded in case of C. tropicum GPCK 512 were relatively superior than C. tropicum GPCK 511.

TABLE 8: Keratinolytic Ability Of Two Different Strains Of Chrysosporium Tropicum AT 0.90 aW Maintained By Using KCl In Shaking Condition

Incubation Period in Days	Fungus Control (ug/ml)	Keratin Control (ug/ml)	Test Sample (ug/ml)	Sum of Keratin and fungus Control (ug/ml)	Net Protein Released (ug/ml)	PH	Weight Loss (%)
CHRYSOSPORIUM TROPICUM GPCK 511							
5	62.5± 3.5	15.0 ±0.0	75.0± 3.5	77.5± 3.5	-2.5	5.2	55.0
10	105.0 ±7.0	17.0 ±0.0	104.5 ± 0.7	122.0 ±7.0	-17.5	5.0	65.0
15	119.5 ±2.1	17.0 ± 0.0	112.0± 2.8	136.5 ±2.1	-24.5	7.0	70.0
20	100.5± 2.1	18.5 ±0.7	122.5 ±2.1	119.0 ± 2.8	3.5 ±0.7	6.7	73.5
25	89.0± 0.0	21.0± 0.0	145.0± 4.2	110.0 ±0.0	35.0±4.2	5.1	78.0
CHRYSOSPORIUM TROPICUM GPCK 512							
5	104.5 ± 0.7	15.0 ± 0.0	124.0 ±1.4	119.5 ±1.4	4.5 ± 0.7	5.2	72.0
10	106.5 ±0.7	17.0 ±0.0	130.5± 0.7	123.5± 3.5	7.0±4.2	5.1	75.2
15	105.5 ±3.5	17.0 ± 0.0	134.5±0.0	122.5 ±3.5	12.0± 4.2	5.1	76.0
20	103.5±0.7	18.5± 0.7	150.5 ±0.7	122.0± 1.4	28.5± 5.0	5.0	80.0
25	91.5 ±2.1	21.0 ±0.0	154.5± 2.1	112.5 ±4.2	42.0 ±2.1	5.4	86.0

The results of protein release in test sample, net protein and percentage weight loss at aw 0.85 by both the strains under static and shaking conditions are reported in Tables 3-4 and Figs. 3-4.

At 0.85 a W the protein release in test sample from hair was as 43.0, 48.0, 130.5, 154.0 and 47.5 ug/ml at 5, 10, 15, 20 and 25 days in the case of C. tropicum GPCK 511 in static condition. The net protein released in culture

filtrate was 3.0, 2.0, 36.5, 48.0 and 4.0 ug/ml at incubation of 5 to 25 days respectively. The values of protein released in test sample were increased up to 20 days. The maximum net protein was noticed at 20 days in static condition. The pH of mineral medium was 5.0 to 5.3 at different incubation periods. Under static condition the weight loss of keratinic substrate was 25.0, 32.5, 68.0, 70.0 and 33.0 per cent at 5 to 25 days of incubation.
The results of C. tropicum GPCK 512 under static condition at the a W 0.85 (KC1) was slightly superior to C. tropicum GPCK 511 when recorded during 5 to 15 days. The protein released in test sample was 62.5, 82.5, 149.5, 100.0 and 95.5 ug/ml in 5, 10, 15, 20 and 25 days. The net protein value increased upto 15 days and later on it was in decreasing trend i.e. 18.5, 34.5, 65.0, 5.0 and 2.5 ug/ml on same incubation period. The pH of medium varied from 5.0 to 5.4 and percentage weight loss was 50.0, 60.5, 79.0, 63.5 and 52.5 per cent at 5, 10, 15, 20 and 25 days respectively.

TABLE 9: Keratinolytic Ability Of Two Different Strains Of Chrysosporium Tropicum At 0.85 aW Maintained By Using KCl In Static Condition

Incubation Period in Days	Fungus Control (ug/ml)	Keratin Control (ug/ml)	Test Sample (ug/ml)	Sum of Keratin and fungus Control (ug/ml)	Net Protein Released (ug/ml)	PH	Weight Loss (%)
CHRYSOSPORIUM TROPICUM GPCK 511							
5	22.0 ±1.4	18.0± 0.0	43.0± 2.8	40.0 ±1.4	3.0 ±1.4	5.0	25.0
10	27.5± 2.1	18.5 ± 0.7	48.0± 2.8	46.0 ±2.1	2.0± 0.7	5.2	32.5
15	74.0 ±1.4	20.0 ±0.0	130.5 ±0.7	94.0± 1.4	36.5± 0.7	5.0	68.0
20	81.0±2.1	25.0± 0.0	154.0 ±1.4	106.0±2.1	48.0± 3.5	5.0	70.0
25	17.5± 0.7	26.0± 0.0	47.5 ±0.7	43.5 ±0.7	4.0± 1.4	5.3	33.0
CHRYSOSPORIUM TROPICUM GPCK 512							
5	26.0 ±1.4	18.0± 0.0	62.5 ± 3.5	44.0± 0.0	18.5 ±3.5	5.2	50.0
10	29.5 ±0.7	18.5± 0.7	82.5 ±3.5	48.0 ±0.0	34.5 ±3.5	5.1	60.5
15	64.5 ±6.3	20.0± 0.0	149.5 ±7.7	84.5 ±1.4	65.0± 1.4	5.0	79.0
20	70.0± 0.0	25.0 ±0.0	100.0± 0.0	95.0±0.0	5.0 ±0.0	5.4	63.5
25	67.0 ±2.8	26.0 ±0.0	95.5 ±3.5	93.0± 2.8	2.5 ± 0.7	5.3	52.5

TABLE 10 : Keratinolytic Ability Of Two Different Strains Of Chrysosporium Tropicum At 0.85 aW Maintained By Using KCl In Shaking Condition.

Incubation Period in Days	Fungus Control (ug/ml)	Keratin Control (ug/ml)	Test Sample (ug/ml)	Sum of Keratin and fungus Control (ug/ml)	Net Protein Released (ug/ml)	PH	Weight Loss (%)
CHRYSOSPORIUM TROPICUM GPCK 511							
5	80.5 ± 0.7	18.5± 0.7	113.5 ±0.7	99.0 ±1.4	14.5± 2.1	6.1	56.0
10	91.5 ± 2.1	19.5± 0.7	156.0 ± 0.7	111.0 ±1.4	45.0± 5.6	5.0	76.0
15	97.0 ± 2.8	21.0± 0.0	130.0± 1.4	118.0 ± 2.8	12.0+ 1.4	6.0	62.0
20	91.0 ± 1.4	26.0± 0.0	120.0± 0.0	117.0 ±1.4	3.0± 4.1	6.2	60.0
25	71.5± 4.9	26.5± 0.7	99.0+ 0.0	98.0±4.2	1.0± 4.9	6.1	52.0
CHRYSOSPORIUM TROPICUM GPCK 512							
5	71.0 ±2.8	18.5± 0.7	104.5 ± 6.3	89.5± 2.1	15.0± 3.4	5.1	53.0
10	73.5 ±2.1	19.5± 0.7	124.5 ± 6.3	93.0± 1.4	31.5± 0.0	5.0	61.5
15	129.5 ±0.7	21.0± 0.0	160.0 ±7.0	150.5± 0.7	9.5± 7.7	5.2	70.0
20	150.0± 0.0	26.0± 0.0	178.5 ±4.9	176.0 ±7.0	2.5±2.1	5.3	80.0
25	112.5 ±0.7	26.5±0.7	162.0 ±2.8	139.0 ± 0.0	23.0± 2.8	5.0	65.0

The keratinolytic activity of C. tropicum GPCK 511 And C. tropicum GPCK 512 was found to be comparatively superior under shaking condition than static conditions at aW 0.85 (KC1). It was found that protein released in test sample increased to its maximum value of 156.0 ug/ml at 10 days of incubation period in case of C. tropicum GPCK 511. At 15, 20 and 25 days of incubation, it was 130.0, 120.0 and 99.0 ug/ml respectively. Whereas maximum pH and percentage weight loss were 6.2 and 76.0 per cent at 20 and 10 days respectively under shaking condition. Whereas C. tropicum GPCK 512 showed increase in protein released in test sample up to 20 days. The protein released in test sample and net protein released was measured as 104.5, 124.5, 160.0, 178.5 and 162.0 ug/ml and 15.0, 31.5, 9.5, 2.5 and 23.0 ug/ml in 5, 10, 15, 20 and 25 days respectively in case of C. tropicum GPCK 512. The pH varied from 5.0 to 5.3 and percentage weight loss was recorded as 53.0, 61.5, 70.0, 80.0 and 65.0 at 5 to 25 days of incubation period.

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