International Refereed Journal of Engineering and Science (IRJES)
ISSN (Online) 2319-183X, (Print) 2319-1821
Volume 11, Issue 5 (Sep.-Oct. 2022), PP. 01-08

An comprehensive analysis of water stress by altering moisture content on keratinolytic ability Of Two Different Strains Of Chrysosporium Tropicum

Dr. Firdos Katiar
Associate Professor, Department of Botany,
Christ Church College, Kanpur, Uttar Pradesh, India

Abstract
Most of the effects of water on cellular functions are closely related to the free energy of water in the cells which determines the water availability for life processes. Water potential is a fundamental concept now widely used in the biological and soil science for quantifying the energy of water in plants, micro-organisms, soil and other related systems (Papendick and Campbell, 1981). Water potential is an abbreviated expression for the 'potential energy of water'. By definition, water potential is the free energy of water in a system relative to the free energy of a reference pool of pure, free water having a specified mass or volume. Water activity (a_w) is a measure of the availability in a substrate of water for microbial growth.
Keywords: keratinophyllic fungi, water stress, fungal growth

I. INTRODUCTION

It is the most important environmental factor influencing growth and colonisation of substrate. Both water activity and water potential are good measures of the amount of water available for microbial growth in a substrate and their relationship has been previously well established (Griffin, 1981).

Much of the work reported has been on pure culture in constant conditions and mainly in osmotically altered liquid or semi-solid media, where liquid water occupies most of the space. There are many environments or niches where fluctuations in water status are frequent, if not regular feature of life.

In microbiological studies, the external osmotic potential is controlled by addition of known concentration of solutes and salts to on agar medium. Various types of solutes and salts have been used to control external osmotic potential including glycerol(Luard, 1983; Magan Cayley and Lacey, 1984), sucrose (Stevens et al, 1983), Glucose, fructose or arabinose (Kushner et al, 1979)NaCl (edgley and brown, (1983). KCl (Fisher, Marasas and Toussoun, 1983 ; Wong 1983, Stapper et al., 1984) as well as a wide variety of other inorganic salts (Jaffee and Zahr, 1983).

Despite of importance of our knowledge of the effect of water availability on the different stages of fungi including some well known pathogens especially keratinophilic fungi is negligible. For a better appreciation of factors affecting disease development, it is essential to know how water availability affects the different stages of reproduction and at what level it is limiting.

II. MATERIALS AND METHODS

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.

The water activities (aw) of this medium was controlled by adding potassium chloride, sodium chloride and sucrose separately in grams/liter as described by Robinson and Stokes, (1955) in the following way :

Water activity (aw)	KC1	NaCl	Sucrose
0.98	44.71	35.1	342
0.95	115.521	86.2875	790.7
0.93	162.102	118.462	1057.80
0.90	231.043	163.8	1431.95
0.85	340.974	235.974	2040.03

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.

III. RESULTS AND DISCUSSION

The results of the effect of moisture level on keratinolytic activity of the two C. tropicum strains (GPCK 511 and GPCK 512) under static and shaking conditions are described in the following.

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.

Addition of sodium chloride as solute in different concentrations in the medium brough out differences in respect of protein release in test sample, net protein and percentage weight loss in static and shaking condition. The results are given in Tables 1-2 and Figs. 1-2.)

At 0.98 aW in which the concentration of sodium chloride was lowest, the protein released from the test sample under static condition varied from 17.5 ug/ml to 51.0 ug/ml recording its maximum value at 20 days of incubation. However, it decreased to 22.5 ug/ml at 25 days of incubation in the case of C. tropicum GPCK 511 511 Net protein released from the test sample also was maximum at 20 days of incubation period. The pattern of net protein released at other period of incubation was very low. The weight loss obtained after the degradation of human hair was found to be 18.0 per cent at 5 days, 19.5 per cent at 10 days, 20.0 per cent at 15 days, 35.0 per cent at 20 days and 21.5 per cent at 25 days. The performance of C. tropicum GPCK 512 was little superior to C. tropicum GPCK 511 as it showed maximum values of 52.0 ug/ml for protein released, 32.5 ug/ml for net protein and 37.0 per cent for weight loss at 15 days of incubation under static condition. The values for these parameters showed decreasing trend at 20 and 25 days. At 0.98 aw pH values in case of both the strains varied from 6.0 to 7.4.

TABLE 1: Keratinolytic Ability Of Two Different Strains Of Chrysosporium Tropicum At 0.98 maintained by using NaCl 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	8.0±0.0	6.0±1.4	17.5±0.7	14.0±1.4	3.5±0.7	6.9	18.0
10	9.5±0.7	7.0±0.0	20.5±0.7	16.5±0.7	4.0±1.4	6.0	19.5
15	11.5±0.7	7.5±0.7	22.0±0.0	19.0±1.4	3.0±0.0	6.8	20.0
20	11.0±2.8	8.0±0.0	51.0±1.4	19.0±2.8	32.0±2.8	6.9	35.0
25	11.0±1.4	9.0±1.4	22.5±2.5	20.0±2.8	2.5±0.7	6.0	21.5
CHRYSOSPORIUM TROPICUM GPCK 512							
5	8.5±0.7	6.0±1.4	28.5±0.7	14.5±0.7	14.0±0.0	7.0	29.0
10	9.5±0.7	7.0±0.0	35.0±1.4	16.5±0.7	18.5±2.1	7.2	30.0
15	12.0±1.4	7.5±0.7	52.0±2.8	19.5±2.1	32.5±4.9	6.9	37.0
20	14.5±0.7	8.0±0.0	28.0±0.0	22.5±0.7	5.5±0.7	6.4	27.3
25	17.0±0.0	9.0±1.4	22.5 0.7	26.0±1.4	-3.5	6.0	24.0

The keratinolytic activity of C. tropicum GPCK 511 and C. tropicum GPCK 512 seemed to be enhanced under shaking condition at the same water activity (0.98) induced by NaCl. Thus protein released from the test sample showed increasing trend with the increasing incubation period. The maximum value was found to be 191.0 ug/ml at 25 days. Similar increasing trend was recorded in weight loss which ranged from 60.0 to 80.0 per cent. However, maximum net protein release was 95.0 ug/ml at 15 days of incubation period in case of C. tropicum GPCK 511 under shaking condition. Almost similar increasing trend, but to a lesser degree was recorded in case of C. tropicum GPCK 512 in respect of protein released and per cent weight loss. The net protein released under shaking condition at 0.98 aW by C. tropicum GPCK 512 showed irregular pattern.

Results of a 0.95 reported in Table 3 clearly indicate poor values of protein released, net protein released and weight loss in case of both the strains. Protein release from test sample by C. tropicum GPCK 511 varied from 14.0 to 21.0 ug/ml.

TABLE 2 : Keratinolytic Ability Of Two Different Strains Of Chrysosporium Tropicum At 0.98 aW Maintained By Using Nac1 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	10.5±0.0	7.5±0.7	73.0±1.4	18.0±1.4	55.0±0.0	6.0	60.0
10	13.0±1.4	8.0±0.0	82.5±3.5	21.0±1.4	61.5±2.1	6.2	62.0
15	25.0±1.4	8.5±0.7	128.5±3.5	33.5±2.1	95.0±5.6	6.0	70.0
20	86.5±0.7	9.0±0.0	164.5±0.7	95.5±6.3	69.0±7.0	6.4	75.0
25	145.5±0.7	9.5±0.7	191.0±1.4	155.0±0.0	36.0±1.4	6.3	80.0
CHRYSOSPORIUM TROPICUM GPCK 512							
5	11.0±1.4	7.0±0.0	50.0±0.0	18.0±2.1	32.0±2.1	6.1	35.0
10	31.5±0.7	8.0±0.0	54.5±0.7	39.5±0.7	15.0±1.4	6.0	39.0
15	37.5±3.5	8.5±0.7	58.5±0.0	46.0±2.8	12.5±2.1	6.2	42.0
20	49.0±1.4	9.0±0.0	62.0±2.8	58.0±1.4	4.0±1.4	6.3	50.5
25	57.0±10.6	9.5±0.7	87.0±2.8	66.5±11.3	20.5±8.5	6.4	64.2

The maximum value of 21.0 ug/ml studied was recorded at 15 days whereas lowest value of 14.0 ug/ml was recorded at 25 days of incubation period. Net protein released was 4.5 ug/ml at 5 days, 3.5 ug/ml at 10 days, -1.5 ug/ml at 15 days, -5.0 ug/ml at 20 days and -5.5 ug/ml at 25 days of incubation period as sum of fungus control and keratin control was more than that of net protein released from test sample. Wheeler et al. (1988) also the growth of some soil inhabiting fungi and their teleomorphs (Aspergilus fleavus, A. penicilliodes, A. wentii and Eurotium rubrum) on different water activities controlled by glucose/fructose media and was partly or highly inhibited by sodium chloride. A. wentii was surprisingly tolerant of sodium chloride at 25°C with high growth rates on both sodium chloride and glucose/fructose media at 0.95 aW. The percentage weight loss in the test sample increased up to 15 days of incubation which decreased at 20 and 25 days of incubation.

The protein released from the test sample in the presence of C. tropicum GPCK 512 under static condition at the same a exhibited the values of 21.0 ug/ml at 5 days, 23.5 ug/ml at 10 days, 20.0 ug/ml at 15 days, 15.0 ug/ml at 20 days and 10.0 ug/ml at 25 days of incubation. The net protein varied from 3.5 to -6.5 ug/ml indicating abrupt change in the behaviour of C. tropicum GPCK 512 towards hair degradation. Maximum percentage weight loss was found to be 20.1 per cent at 10 days of incubation. The pH recorded after different incubation period at this water activity showed comparatively larger variations from 5.0 to 7.0.

TABLE 3: Keratinolytic Ability Of Two Different Strains Of Chrysosporium Tropicum at 0.95 aW maintained by using NacCl 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	6.5±2.1	6.5±0.7	17.5±0.7	13.0±1.4	4.5±0.7	5.5	13.9
10	8.5±0.7	7.0±1.4	19.0±0.0	15.5±2.1	3.5±2.1	7.0	17.4
15	14.0±1.4	8.5±0.7	21.0±1.4	22.5±0.7	-1.5	6.0	18.7
20	11.0±1.4	9.0±0.0	15.0±0.0	20.0±1.4	-5.0	5.0	10.0
25	10.0±0.0	9.5±0.7	14.0±0.0	19.5±0.7	-5.5	5.0	12.1
CHRYSOSPORIUM TROPICUM GPCK 512							
5	11.0±0.0	6.5±0.7	21.0±0.0	17.5±0.7	3.5±0.7	5.0	19.4
10	18.0±0.0	7.0±1.4	23.5±0.7	25.0±1.4	-1.5	7.0	20.1
15	10.0±0.0	8.5±0.0	20.0±0.0	18.5±0.7	1.5±0.7	6.0	19.4
20	9.0±0.0	9.0±0.0	15.0±0.0	18.0±0.0	-3.0	6.2	14.0
25	7.0±0.0	9.5±0.7	10.0±0.0	16.5±0.7	-6.5	6.1	9.0

The keratinolytic activity of both the strains at the same water activity was found to be superior in shaking condition as compared to static condition (Table 4 and Fig. 1). Thus protein released the test sample showed regular increase from 67.0 ug/ml to 124.5 ug/ml during 5 days to 25 days of incubation period in the case of C. tropicum GPCK 511. Net protein released showed increasing trend up to 15 days of incubation thereafter it decreased. Weight loss in human hair also showed increase with the increase in incubation period recording maximum per cent weight loss at 25 days of incubation (Fig.2).

Under the similar set of reaction conditions the ability of C. tropicum GPCK 512 was found to be overall superior than C. tropicum GPCK 511 as it favoured more protein release in test sample, net protein release and per cent weight loss from the test sample. There was an over all and gradual increase from 15 days to 25 days of incubation. Maximum values of 152.0 ug/ml, 93.0 ug/ml and 69.5 per cent were recorded at 25 days of incubation period in protein release in test sample, net protein and per cent weight loss respectively.

TABLE 4: Keratinolytic Ability Of Two Different Strains Of Chrysosporium Tropicum At 0.95 aW maintained by using NaCl 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	45.5±0.7	7.0±0.0	67.0±1.4	52.5±0.7	14.5±2.1	5.0	54.9
10	57.0±1.4	7.5±0.7	85.5±6.3	64.5±0.7	21.0±7.0	5.9	52.0
15	71.0±1.4	9.0±1.4	114.5±2.1	80.0±2.8	34.5±0.7	6.3	57.9
20	77.5±3.5	10.0±0.0	117.0±0.0	87.5±3.5	29.5±29.5	5.1	59.0
25	102.5±3.5	10.5±0.7	124.5±7.4	113.0±2.8	11.5±10.6	5.3	60.0
CHRYSOSPORIUM TROPICUM GPCK 512							
5	22.0±1.4	7.0±0.0	31.0±1.4	29.0±1.4	2.0±0.0	5.2	25.0
10	27.5±0.7	7.5±0.7	38.5±2.7	35.0±0.0	3.5±2.1	5.6	29.0
15	29.0±1.4	9.0±1.4	57.5±3.5	38.0±2.8	19.5±6.3	6.0	45.0
20	32.5±3.5	10.0±0.0	129.5±5.0	42.5±3.5	87.0±0.0	5.3	66.0
25	48.5±4.9	10.5±0.7	152.0±2.8	59.0±4.2	93.0±1.4	6.2	69.5

The results at 0.93 aW under static and shaking conditions reported in Tables 5-6 and Fig. 2. At 0.93 aw gradual rise in protein level in the test sample were recorded in static condition. The maximum values of protein released in test sample was 23.0 ug/ml at 25 days of incubation period. The net protein values were 0.5, 1.0, 2.5, 0.5 and 2.5 ug/ml at 5, 10, 15, 20 and 25 days respectively in case of C. tropicum GPCK 511. The weight loss was 10.5, 12.0, 15.0, 16.5 and 20.5 per the same incubation periods. The The performance of C. tropicum GPCK 512 was almost same to C. tropicum GPCK 511, it showed maximum values of 20.5 ug/ml for protein released, 4.0 ug/ml for net protein released and 14.1 per cent for weight loss at 20, 25 and 20 days respectively under static condition. The pH values in both the strain varied from 5.0 to 6.0. 25 days of incubation period. The net protein value was found to be 5.5, 10.0, 11.5, 84.5 and 20.5 ug/ml and -1.5, -1.0, -2.5, 0.0 and 14.5 ug/ml in both the strains. The pH varied from 5.0 to 6.2 in the case of C. tropicum GPCK 511 and 5.0 to 5.8 in C. tropicum GPCK 512. The percentage weight loss under shaking condition (Table 6 and Fig. 2) was 19.0, 22.5, 30.0, 68.2 and 70.0 in case of GPCK 511 strain and 10.5, 11.2, 14.0, 20.0, and 30.5 in GPCK 512 strain at 5, 10, 15, 20 and 25 days respectively.

TABLE 5: Keratinolytic Ability Of Two Different Strains Of Chrysosporium Tropicum At 0.93 Maintained By Using NaCl 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	7.5±0.7	4.5±0.7	12.5± 1.4	12.0± 0.7	0.5± 0.7	6.0	10.5
10	8.0± 0.0	6.0± 0.0	15.0± 1.4	14.0±0.0	1.0±1.4	5.0	12.0
15	9.5± 0.7	6.5± 0.7	18.5± 0.0	16.0±0.7	2.5±0.7	5.0	15.0
20	10.0± 1.4	6.5±0.7	17.0± 0.0	16.5±0.7	0.5±0.7	5.1	16.5
25	13.5±0.7	7.0±0.0	23.0±1.4	20.5±0.7	2.5±2.1	5.3	20.5
CHRYSOSPORIUM TROPICUM GPCK 512							
5	7.0±0.0	5.0±0.7	14.5± 0.7	12.0±0.7	2.5± 0.7	5.2	6.0
10	9.0±0.0	6.0±0.0	18.0±0.7	15.0±0.0	3.0± 0.0	5.1	9.5
15	10.5±0.7	6.5±0.7	20.0±0.7	17.0±0.0	3.0± 0.0	5.2	10.5
20	11.5± 0.7	6.5± 0.7	20.5±0.0	18.0± 1.4	2.5±1.4	5.3	14.1
25	5.0±0.7	7.0±0.0	16.0±0.0	12.0±0.0	4.0± 0.0	5.0	6.0

TABLE 6: Keratinolytic Ability Of Two Different Strains Of Chrysosporium At Tropicum 0.93 aW Maintained By Using NaCl 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	6.0±0.0	6.5±0.0	18.0±1.4	12.5±0.7	5.5±0.7	5.0	19.0

10	10.5±0.7	7.0±0.0	27.5±3.5	17.5±0.7	10.0±2.8	5.2	22.5
15	13.0±1.4	7.5±0.7	32.0±4.2	20.5±2.1	11.5±2.1	5.5	30.0
20	27.5±2.1	8.0±0.0	120.0±32.4	35.5±2.1	84.5±0.7	5.8	68.2
25	104.5±6.3	8.5±0.7	133.5±3.5	113.0±5.6	20.5±9.1	6.2	70.0
CHRYSOSPORIUM TROPICUM GPCK 512							
5	8.5±0.7	6.5±0.0	13.5±0.7	15.0±5.6	-1.5	5.2	10.5
10	9.5±0.7	7.0±0.0	15.5±0.7	16.5±8.7	-1.0	5.0	11.2
15	12.0±1.4	7.5±0.7	17.0±0.0	19.5±2.1	-2.5	5.3	14.0
20	14.0±1.4	8.0±0.0	22.0±2.8	22.0±1.4	0.0	5.1	20.0
25	16.5±2.1	8.5±0.7	39.5±0.7	25.0±2.8	14.5±2.1	5.8	30.5

Chrysosporium tropicum GPCK 611
A-KCI
B-N&CI
C-SUCROSE

Chrysosporium troploum GPCK 612
D-KCI
E-NaCl
F-SUCROSE

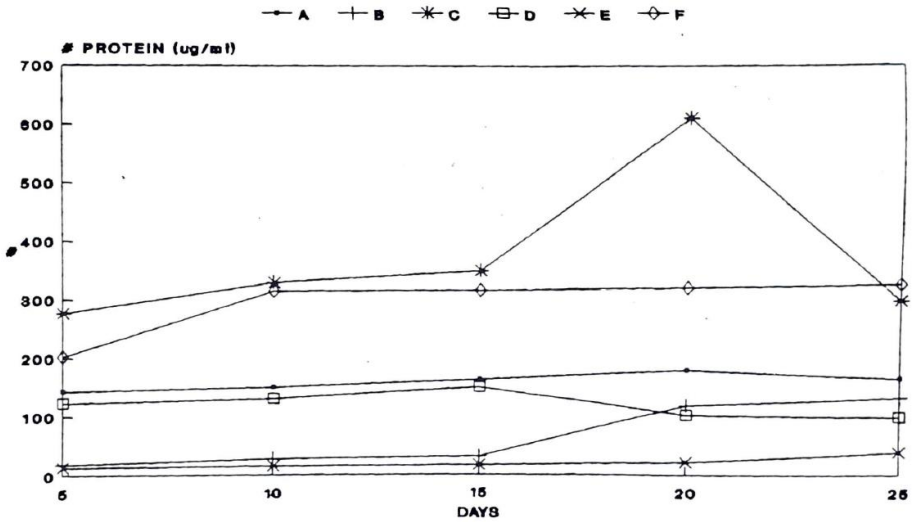

FIG. 1. Protein Released In The Culture Filtrate Of Chrysosporium Tropicum GPCK 511 And Chrysosporium Tropicum GPCK 512 At 0.93 Aw Controlled By KCl, NaCl AND Sucrose In Shaking Condition Using Human Hair.

Chrysosporium tropicum GPCK 611
A-KCI
B-N&CI
C-SUCROSE

Chrysosporium troploum GPCK 612
D-KCI
E-NaCl
F-SUCROSE

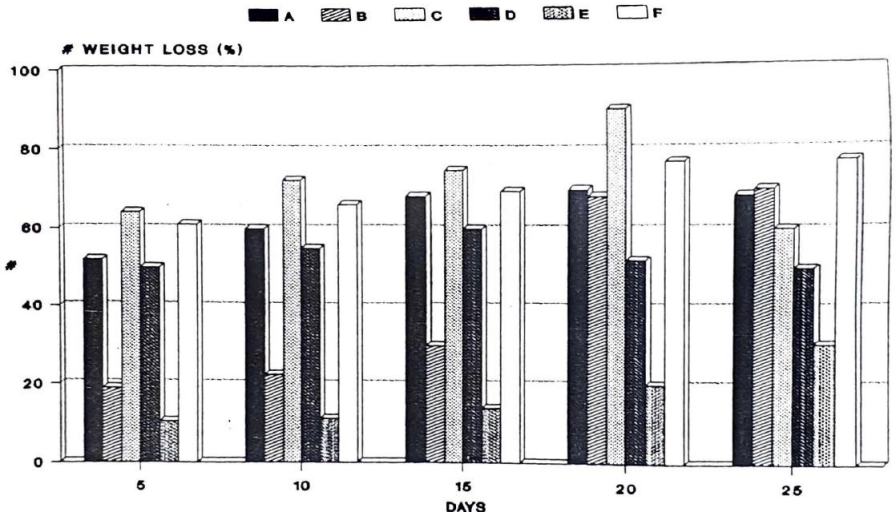

FIG. 2. Weight Loss Of Human Hair As Indused By Chrysosporium Tropicum GPCK 511 AND Chrysosporium Tropicum GPCK 512 AT 0.93 aw Controlled By KCl, NaCl AND Sucrose In Shaking Condition.

At 0.90 a W controlled by NaCl, the protein released from the test sample under static condition, it varied from 19.0 to 61.0 ug/ml recording its maximum value at 25 days of incubation period in the case of C. tropicum GPCK 511. The net protein values were 4.0, 4.0, 4.5, 4.0 and 34.0 ug/ml. The mximum value of net protein was 34.0 ug/ml at 25 days. The weight loss of keratin in mineral medium was 13.5, 20.0, 22.5, 27.0 and 49.0 per cent at 5, 10, 15, 20 and 25 days in case of C. tropicum GPCK 511 (Table 7)..

The results of C. tropicum GPCK 512 showed less degradation of human hair as compared to C. tropicum GPCK 511. The values of protein released in test sample were 16.0, 20.0, 22.0, 25.5 and 30.0 ug/ml at different incubation periods.

TABLE 7 : Keratinolytic Ability Of Two Different Strains Of Chrysosporium Tropicum AT 0.90aW Maintained By Using NaCl 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	8.0±0.0	7.0±0.0	19.0±1.4	15.0±0.0	4.0±1.4	5.6	13.5
10	9.5±0.7	9.0±0.0	22.5±0.7	18.5±0.7	4.0±0.0	5.2	20.0
15	11.0±1.4	9.5±0.7	25.0±1.4	20.5±2.1	4.5±5.1	5.1	22.5
20	14.5±0.7	10.0±0.0	28.5±2.1	24.5±0.7	4.0±2.8	5.9	27.0
25	16.5±0.7	10.5±0.7	61.0±1.4	27.0±1.4	34.0±0.0	5.3	49.0
CHRYSOSPORIUM TROPICUM GPCK 512							
5	8.0±0.0	7.0±0.0	16.0±0.0	15.0±0.0	1.0±0.0	5.0	11.2
10	10.0±0.0	9.0±0.0	20.0±0.0	19.0±0.0	1.0±0.0	5.1	22.2
15	11.0±0.0	9.5±0.7	22.0±0.0	20.5±0.7	1.5±0.7	5.2	24.4
20	12.5±0.7	10.0±0.0	25.5±0.7	22.5±0.7	3.0±0.0	5.0	22.8
25	16.0±0.0	10.5±0.7	30.0±0.0	26.5±0.7	3.5±0.7	5.0	33.3

The pattern of net protein released at different incubation periods was very low and it was recorded as 1.0, 1.0, 1.5, 3.0 and 3.5 ug/ml at 5, 10, 15, 20 and 25 days respectively. The maximum pH and percentage weight loss was recorded 5.2 and 33.3 per cent 15 and 25 days respectively.

The keratinolytic activity of both the strains at the same water activity was found to be greater in shaking conditions as compared to static condition. The protein released in test sample showed increasing trend up to 20 days of incubation thereafter it decreased. The maximum value of protein released in test sample was 172.5 ug/ml at 20 days, maximum net protein was 144.5 ug/ml at 20 days of incubation in the case of C. tropicum GPCK 511 (Table 8, Fig. 2). The pH of medium varied from 5.0 to 6.3 and maximum percentage weight loss was 78.8 at 20 days of incubation.

In case of C. tropicum GPCK 512 the value of protein released in test sample showed increasing trend with the increasing incubation period. The maximum value was found to be 75.5 ug/ml at 25 days. Weight loss ranged from 12.8 to 53.4 per cent (Fig. 2). Net protein release was maximum (24.5 ug/ml) at 25 days of incubation period under shaking condition. The pH was

TABLE 8 : Keratinolytic Ability Of Two Different Strains Of Chrysosporium Tropicum At 0.90aW Maintained By Using Nac1 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	7.5±0.7	8.0±1.4	18.5±0.7	15.5±2.1	3.0±1.4	5.0	12.9
10	9.5±0.7	9.0±0.0	30.0±0.0	18.5±0.7	11.5±0.7	5.2	22.7
15	12.0±1.4	9.5±0.7	42.5±3.5	21.5±2.1	21.0±1.4	5.1	28.9
20	18.0±5.0	10.0±0.0	172.5±2.8	28.0±0.7	144.5±2.1	6.0	78.8
25	31.5±0.5	10.5±0.7	116.5±2.1	42.0±1.4	74.5±0.7	6.3	59.7
CHRYSOSPORIUM TROPICUM GPCK 512							
5	14.5±0.7	8.0±1.4	18.0±1.4	22.5±2.1	-4.5	5.1	12.8
10	17.0±0.0	9.0±0.0	20.5±0.7	26.0±0.0	-5.5	5.0	19.9
15	20.0±0.0	9.5±0.7	24.0±0.7	29.5±0.7	-5.5	5.3	22.9
20	32.5±3.5	10.0±0.0	34.5±0.7	42.5±3.5	-8.0	6.1	33.2
25	40.5±2.	10.5±0.7	75.5±3.5	51.0±1.4	24.5±4.9	6.2	53.4

5.1. 5.0, 5.3, 6.1 and 6.2 per cent at 5, 10, 15, 20 and 25 days of incubation periods.

The results of at 0.85 aw by both the strains under static and shaking condition were shown in Tables 9-10 and Fig. 2. At 0.85 aW the net protein released in the culture filtrate from hair was 4.0, 3.5, 3.5, -3.0 and -5.0 ug/ml at 5, 10, 15, 20 and 25 days respectively in static condition in the case of C. tropicum GPCK 511. The protein released in the test sample was 20.5, 23.0, 26.0, 27.5 and 16.0 ug/ml at incubation of 5 to 25 days respectively. Whereas C. tropicum GPCK 512 showed increase in protein release in test sample up to 20 days of incubation period. The protein released in test sample and net protein released were 12.0, 24.5, 26.5, 41.5 and 19.0 ug/ml and -4.5, -1.5, 0.0, 18.0 and -3.5 ug/ml in 5, 10, 15, 20 and 25 days respectively.

In C. tropicum GPCK 511 the pH of mineral media was 5.0, 5.2, 5.1, 5.0 and 5.1 at the same incubation period while in C. tropicum GPCK 512, the pH was 5.2, 5.1, 5.2, 5.0 and 5.1 in 5, 10, 15, 20 and 25 days of incubation period. The maximum percentage weight loss was 28.8 at 25 days in case of C. tropicum GPCK 511) and 37.1 per cent at 20 days in the case of C. tropicum GPCK 512.

***TABLE 9: Keratinolytic Ability Of Two Different Strains Of Chrysosporium Tropicum At 0.85 aW Maintained By Using NaCl 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	9.0±0.0	7.5±0.7	20.5±0.7	16.5±0.7	4.0±1.4	5.0	18.5
10	11.5±0.7	8.0±1.4	23.0±1.4	19.5±0.7	3.5±2.1	5.2	27.0
15	13.5±2.1	9.0±0.0	26.0±0.0	22.5±2.1	3.5±2.1	5.1	28.8
20	20.5±0.7	10.0±1.4	27.5±0.7	30.5±0.7	-3.0	5.0	27.9
25	10.0±0.0	11.0±0.0	16.0±1.4	21.0±0.0	-5.0	5.1	14.6
CHRYSOSPORIUM TROPICUM GPCK 512							
5	9.0±0.0	7.5±0.0	12.0±0.0	16.5±0.0	-4.5	5.2	7.0
10	15.0±0.0	8.0±1.4	24.5+ 0.7	23.0±0.0	-1.5	5.1	24.0
15	17.5±0.7	9.0±0.0	26.5+ 0.7	26.5±0.0	0.0	5.2	29.2
20	13.5±0.7	10.0±1.4	41.5±2.1	23.5±2.1	18.0±0.0	5.0	37.1
25	11.5±0.7	11.0±0.0	19.0±0.0	22.5±0.7	-3.5	5.1	15.7

In shaking condition at the same aW both the strains caused degradation in superior way in respect of protein released in test sample and percentage weight loss. The protein released in test sample was 28.0, 52.0, 57.0, 58.5 and 61.5 ug/ml and net protein value was 3.0, 19.5, 21.0, 17.5 and 18.0 ug/ml at 5, 10, 15, 20 and 25 days respectively in case of C. tropicum GPCK 511. The pH of medium was 5.1, 5.0, 5.0, 5.3 and 5.7. The maximum weight loss was 59.9 per cent at 25 days of incubation in case of C. tropicum GPCK 511 (Fig. 2).)

Almost similar increasing trend was shown by C. tropicum GPCK 512 in respect of protein released and percentage weight loss. The net protein released under shaking condition at 0.85 a by C. tropicum GPCK 512 was 10.0, 11.5, 10.0, 27.5 and 25.5 ug/ml in 5, 10, 15, 20 and 25 days respectively. The maximum pH and weight loss was 5.4 and 56.0 per cent at 15 and 25 days respectively.

References:

[1]. Huq, C. A. Whitehouse, C. J. Grim, M. Alam and R. R. Colwell, Curr. Opin. Biotechnol., 2008, 19, 244–247.
[2]. F. Emtiazi, T. Schwartz, S. M. Marten, P. Krolla-Sidenstein and U. Obst, Water Res., 2004, 38, 1197–1206.
[3]. L. Hall-Stoodley and P. Stoodley, Trends Microbiol., 2005, 13, 7–10.

[4]. M. Momba, R. Kfir, S. N. Venter and T. E. Cloete, Water SA, 2000, 26, 59–66.
[5]. L. C. Simo˜es, M. Simo˜es and M. J. Vieira, Water Science & Technology: Water Supply, 2012, 12(3), 334–342.
[6]. G. C. Whipple, Journal of the New England Water Works Association, 1897, 12, 1–19.
[7]. S. C. Prescott and C. E. A. Winslow, in Elements of water bacteriology, J. Wiley & Sons, New York, 1904.
[8]. J. T. Walker and M. Morales, Water Sci. Technol., 1997, 35, 319–323.
[9]. R. T. Bachmann and R. G. J. Edyvean, Biofilms, 2005, 2, 197–227.
[10]. S. Skraber, J. Schijven, C. Gantzer and A. M. de RodaHusman, Biofilms, 2005, 2, 105–117.
[11]. H. Olson, R. McCleary and J. Meeker, in Modeling the Environmental Fate of Microorganisms, ed. C. J. Hurst, American Society for Microbiology, Washington, DC, 1991, pp. 255–285.
[12]. J.-C. Block, M. Dutang, J. Maillard and D. Reasoner, Water Supply, 1994, 12, SS1/8–SS1/12.
[13]. D. van der Kooij and H. R. Veenendaal, Water Supply, 1994, 12, SS1/1–SS1/7.
[14]. K. Camper, M. Burr, B. Ellis, P. Butterfield and C. Abernathy, J. Appl. Microbiol., 1998, 85, 1S–12S.
[15]. J. T. Walker, S. Ives, M. Morales, N. L. Pavey and A. A. West, Int. Biodeterior. Biodegrad., 1997, 39, 88–89.
[16]. D. van der Kooij, J. H. M. van Lieverloo, J. A. Schellart and P. Hiemstra, Journal of Water Services Research and Technology – Aqua, 1999, 48, 31–37.
[17]. M. A. Shannon, P. W. Bohn, M. Elimelech, J. G. Georgiadis, B. J. Marin˜as and A. M. Mayes, Nature, 2008, 452, 301–310.
[18]. S. D. Kim, J. Cho, I. S. Kim, B. J. Vanderford and S. A. Snyder, Water Res., 2007, 41, 1013–1021.
[19]. B. Kasprzyk-Hordern, R. M. Dinsdale and A. J. Guwy, Water Res., 2008, 42, 3498–3518.
[20]. E. Hrudey and E. J. Hrudey, in Lessons from recent outbreaks in affluent nations, International Water Association Publishing, London, 2004.
[21]. M. F. Craun, G. F. Craun, R. L. Calderon and M. J. Beach, J. Water Health, 2006, 4, 19–30.
[22]. P. Beaudeau, H. de Valk, V. Vaillant, C. Mannschott, C. Tillier, D. Mouly and M. Ledrans, J. Water Health, 2008, 6, 491–503.
[23]. M. F. Blasi, M. Carere, M. G. Pompa, E. Rizzuto and E. Funari, J. Water Health, 2008, 6, 423–432.
[24]. P. Karanis, C. Kourenti and H. Smith, J. Water Health, 2007, 5, 1–38.
[25]. S. P. Payment, Can. J. Microbiol., 1999, 45, 709–715.
[26]. B. Barbeau, P. Payment, J. Coallier, B. Cle´ment and M. Pre´vost, Quant. Microbiol., 2000, 2, 37–54.
[27]. L. Gofti-Laroche, D. Demanse, J. C. Joret and D. Zmirou, J. Am. Water Works Assoc., 2003, 95, 162–172.
[28]. L. Gofti-Laroche, B. Gratacap-Cavallier, D. Demanse, O. Genoulaz, J. M. Seigneurin and D. Zmirou, J. Clin. Virol., 2003, 27, 74–82.
[29]. M. Exner, Hygiene + Medizin, 2004, 29, 418–4227.
[30]. S. Glaberman, J. E. Moore, C. J. Lowery, R. M. Chalmers, I. Sulaiman, K. Elwin, P. J. Rooney, B. C. Millar, J. S. Dooley, A. A. Lal and L. Xiao, Emerging Infect. Dis., 2002, 8, 631–633.
[31]. M.-L. Hanninen, H. Haajanen, T. Pummi, K. Wermundsen, M.-L. Katila, H. Sarkkinen, I. Miettinen and H. Rautelins, Appl. Environ. Microbiol., 2003, 69, 1391–1396.
[32]. B. Said, F. Wright, G. L. Nichols, M. Reacher and M. Rutter, Epidemiol. Infect., 2003, 130, 469–479.
[33]. T. V. Amvrosy'eva, Z. F. Bogush, O. N. Kazinets, O. V. Dyakonova, N. V. Poklonskaya, G. P. Golovnyova and R. M. Sharko, Vopr. Virusol., 2004, 49, 30–34.
[34]. R. Laporte, P. Pernes, P. Pronni, F. Gottrand and P. Vincent, Br. Med. J., 2004, 329, 204–205.
[35]. L. Maunula, I. T. Miettinen and C. H. Von Bonsdorff, Emerging Infect. Dis., 2005, 11, 1716–1721.
[36]. X. Garg, J. Marshall, M. Salvadori, H. R. Thiessen- Philbrook, J. Macnab, R. S. Suri, R. B. Haynes, J. Pope and W. Clark, on behalf of the Walkerton Health Study Investigators, J. Clin. Epidemiol., 2006, 59, 421–428.
[37]. J. Empel, K. Filczak, A. Mrowka, W. Hryniewicz, D. A. Livermore and M. Gniadkowski, J. Clin. Microbiol., 2007, 45, 2829–2834.
[38]. J. Hewitt, D. Bell, G. C. Simmons, M. Rivera-Aban, S. Wolf and G. E. Greening, Appl. Environ. Microbiol., 2007, 73, 7853–7857.
[39]. G. F. Craun, J. M. Brunkard, J. S. Yoder, V. A. Roberts, J. Carpenter, T. Wade, R. L. Calderon, J. M. Roberts, M. J. Beach and S. L. Roy, Clin. Microbiol. Rev., 2010, 23, 507–528.
[40]. H. M. L. Kvitsand and L. Fiksdal, Water Sci. Technol., 2010, 61, 563–571.
[41]. B. G. Blackburn, G. F. Craun, J. S. Yoder, V. Hill, R. L. Calderon, N. Chen, S. H. Lee, D. A. Levy and M. J. Beach, MMWR Surveill. Summ., 2004, 53, 23–45.