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Protein Released In the Culture Filtrate Of two selected strain of Chrysosporium Tropicum Controlled by KCl, NaCl AND sucrose in shaking condition using human hair

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Abstract
Sucrose is one of the most important component of nutrient media upon which mycelium of the fungi maintains its growth. Proper growth of fungi leads to the increase rate of keratinolytic ability of the fungal strains. It was therefore planed to determine optimum doses of sucrose at which the keratinolytic activity of the C. tropicum GPCK 511 and GPCK 512 are maximum. The experiment performed using increasing doses of sucrose created water stress conditions and lowered water activities. The results on keratinolytic activity of C. tropicum strains GPCK 511 and GPCK 512 at different water activities due to sucrose applications are analysed here.
Keywords: ***keratinophyllic fungi, water stress, fungal growth***

I. Introduction

The ability of keratinophilic fungi and dermatophytes to decompose and parasitise keratinous substrate is closely associated with the depends upon the utilization of keratin. Fungi capable of colonizing keratinous substrates such as human hair, skin, feathers, hooves, horns, and nails are widely spread in nature. Among the multitude of fungal species known to colonize keratin, some are able to parasitise men and animals, causing diseases (Gentles, 1962; Singh, 1969; Hubalek and Hornick, 1977). These keratinophilic fungi include genera Chrysosporium, Trichophyton, Epidermophyton, Microsporum , Myceliophthora, Malbranchea and their teleomorphs.

Among keratinophilic fungi, genus Chrysosporium is one of the most important fungus having ability to degrade keratinous substrates to higher degree as compared to others. However, work on its ability to degrade kertain is limited and requires further exploration. The affinity of C. tropicum for keratinic substrates has been shown in numerous isolations from soil. Its capacity of utilize kertain has been shown by Jain and Agrawal. (1980).

Kushwaha (1983) and Nigam and Kushwaha, (1992a) Its pathogenic potential has been demonstrated by Hubalek and Hornick (1977). The capacity of the fungus to attack autoclaved human hair with the aid of penetrating bodies has been described by Carmichael (1962). Moreover Fillipello Marchisio (1986) was able to demonstrate several types of surface erosion and radial penetration of fungal organs in the keratin substrates through light microscopy. Scanning electron microscopy of human hair revealed its biodeterioration and longitudinal furrows were caused by Myceliophthora anamorph of Arthroderma tuberculatum. Development of deeper furrows in hair indicated the rapid degrability of the fungus (Nigam and Kushwaha 1990). Human hair deterioration in humid chamber was also studied by (Mercer and Verma, 1933; Baxter and Mann, 1969; Hsu and Volz, 1975 and Kunert and Krajci, 1981). Bahuguna and Kushwaha (1989) applied in vitro hair perforation test to eight species of Chrysosporium and one species each of Microsporum and Trichophyton. Six of them were found to perforate hair while four were negative.)

TABLE 1: Keratinolytic Ability Of Two Different Strains Of Chrysosporium Tropicum At 0.85 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	13.0±1.4	12.0±0.0	28.0±0.0	25.0±1.4	3.0±1.4	5.1	27.9
10	18.5±0.7	14.0±0.0	52.0±2.8	32.5±0.7	19.5±2.1	5.0	47.8
15	21.0±1.4	15.0±1.4	57.0±1.4	36.0±2.8	21.0±1.4	5.0	48.1
20	25.0±1.3	16.0±0.0	58.5±0.7	41.0±2.8	17.5±2.1	5.3	58.2
25	27.0±1.4	16.5±0.7	61.5±2.1	43.5±0.7	18.0±2.8	5.7	59.9
CHRYSOSPORIUM TROPICUM GPCK 512							
5	5.0±0.0	12.0±0.0	27.0±1.4	17.0±0.0	10.0±1.4	5.0	22.9

10	10.5±0.7	14.0±0.0	36.0±2.8	24.5±2.1	11.5±0.7	5.2	33.8
15	14.0±1.4	15.0±1.4	39.0±1.4	29.0±0.7	10.0±0.7	5.4	43.5
20	19.0±1.4	16.0±0.0	62.5±9.1	35.0±7.7	27.5±0.7	5.4	54.6
25	24.0±1.4	16.5±0.7	66.0±4.2	40.5±2.8	25.5±2.8	5.1	56.0

At the 0.98 a W maintained by using sucrose in static condition, C. tropicum GPCK 511 showed increasing trend in protein release in test sample, net protein released, pH and percentage weight loss up to 15 days of incubation thereafter it decreased at 20 and 25 days of incubation. The maximum values for protein release in test sample, net protein released and weight loss were recorded to be 149.5 ug/ml, 54.5 ug/ml and 72.0 per cent respectively.

On the other hand C. tropicum GPCK 512 could show enhanced activity in the same reaction conditions and at the same water activity as revealed by higher values of protein released which continuously increased up to 20 days of incubation to the extent of 369.5 ug/ml. However, it decreased to 194.5 ug/ml at 25 days of incubation. Weight loss also exhibited increasing trend up to 20 days thereafter it decreased at 25 days of incubation. Maximum weight loss reached to 89.9 per cent indicating enhanced ability of C. tropicum GPCK 512 at the water activity of 0.98 (sucrose). The pH did not show much variations under the static condition at this a however, it varied from 6.4 to 7.0 in case of both the strains.

TABLE 2: Keratinolytic Ability Of Two Different Strains Of Chrysosporium Tropicum At 0.98 aW Maintained By Using Sucrose 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	19.5±0.7	7.0± 0.0	55.5±4.9	26.5± 0.7	29.0±4.2	7.0	21.1
10	24.5±2.1	17.0±1.4	78.0±2.8	41.5±0.7	36.5±3.5	6.9	61.9
15	74.0±1.4	21.0±1.4	149.5±0.7	95.0±0.0	54.5±0.7	7.0	72.0
20	70.2±2.1	25.0±0.0	141.5±0.7	95.0±0.7	46.5±2.8	6.7	71.8
25	62.0± 1.4	26.0±1.4	95.0±4.2	88.0±2.8	7.0±7.0	6.5	63.1
CHRYSOSPORIUM TROPICUM GPCK 512							
5	176.0±2.8	7.0±0.0	221.5±2.1	183.0±2.8	38.5±4.5	6.7	73.1
10	191.0±1.4	17.0± 1.4	247.0±2.8	208.0±2.8	39.0±5.6	6.9	73.9
15	321.0±8.4	21.0±1.4	350.0± 0.0	342.0±7.0	8.0±7.0	6.4	83.5
20	260.5±0.7	45.0±0.0	369.5±0.7	305.5±0.7	64.0±1.4	6.6	89.9
25	133.0±4.2	46.5±2.1	194.5± 6.3	179.5±2.1	15.0±8.4	6.9	62.2

The keratinolytic ability at water activity of 0.98 (sucrose) in shaking condition showed superior values for protein release in test sample, net protein and percentage weight loss as compared to static condition. Protein released in test sample increased regularly up to 15 days of incubation showing the values of 363.0 ug/ml, thereafter it decreased to 283.5 and 204.5 ug/ml at 20 and 25 days of incubation in case of C. tropicum GPCK 511. Maximum value of 194.0 ug/ml in respect of net protein released and 82.1 per cent in respect of weight loss were recorded at 15 days of incubation period.

C. tropicum GPCK 512 again proved more beneficial at 0.98 aW as compared to C. tropicum GPCK 511 showing linear increase in protein release up to 20 days of incubation period and

TABLE 3: Keratinolytic Ability Of Two Different Strains Of Chrysosporium At Tropicum 0.98 aW Maintained By Using Sucrose 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	53.0±5.6	10.0±0.0	72.5±3.5	63.0±5.6	9.5±9.1	7.0	41.4
10	83.5±2.1	21.0±1.4	278.0±2.8	104.5±3.5	173.5±6.3	7.1	72.7
15	146.0±5.6	23.0±1.4	363.0±4.2	169.0±4.2	194.0±0.0	6.9	82.1
20	140.5±0.7	29.0±0.0	283.5±2.1	169.5±0.7	114.0±2.8	6.8	72.0
25	105.0±5.6	30.0±0.0	204.5±5.6	135.0±5.6	69.5±0.7	7.2	70.9
CHRYSOSPORIUM TROPICUM GPCK 512							
5	205.0±0.7	10.0±0.0	236.5±4.9	215.0±7.0	21.5±1.7	6.0	74.5
10	231.5±9.1	21.0±1.4	282.5±3.5	252.5±7.3	30.0±11.3	6.1	75.0
15	333.0±4.2	23.0±1.4	377.0±2.8	356.0±2.8	21.0±5.6	6.3	85.2
20	335.0±5.6	29.0±0.0	379.0±0.7	364.0±5.6	15.0±6.3	6.2	85.9
25	340.5±7.7	30.0±0.0	367.5±3.5	370.5±3.5	-3.0	6.0	90.8

maximum value of 379.0 ug/ml. Net protein release suffered a set back giving 21.5 ug/ml as maximum value. Maximum weight loss of 90.8 per cent was obtained at 25 days of incubation. Magan and Lacey (1948a, b) also studied the effect of temperature and pH on water relations of field and storage fungi and few plant pathogenic fungi i.e. Fusarium avenaceum, F. culmorum, F. Poae and F. tricinctum and found that the optimum and minimum aW for all species except F. poae were 0.95-0.99 and 0.89-0.90 at optimum temperatures. Cuero et al., (1987) studied the effect of water activity, temperature and substrate on mycotoxin production by A. flavus, Penicillium viridicatum and F. graminearum and recorded the highest mycotoxin production at 0.98 and 0.95 aw
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The keratinolytic ability of C. tropicum GPCK 511 under static condition at 0.95 aW showed increasing trend (Table 4) in respect of protein release in test sample, net protein released and percentage weight loss up to 15 days incubation period, thereafter, it showed decreasing trend at 20 and 25 days of incubation. The maximum values recorded were 381.0 ug/ml, 111.0 ug/ml and 83.3 per cent for protein release in test sample, net protein released and weight loss respectively.

In the case of C. tropicum GPCK 512 similar increasing trend up to 15 days of incubation was recorded for protein released in test sample and weight loss. However, maximum value of net protein released was 114.0 ug/ml at 20 days incubation period under static condition (Table 5).

TABLE 4: Keratinolytic Ability Of Two Different Strains Of Chrysosporium Tropicum AT 0.95aW Maintained By Using Sucrose 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	197.5±2.1	30.5±0.7	291.5±2.1	228.0 1.4	63.5±0.7	7.1	72.5
10	201.0±1.4	32.5±3.5	296.0±2.8	233.5±2.1	62.5±4.9	7.9	82.8
15	233.0±4.2	37.0±1.4	381.0±1.4	270.0±5.6	111.0±7.0	7.8	83.3
20	164.0±5.6	39.0±1.4	272.5±0.7	203.0±4.2	69.5±4.9	7.2	73.1
25	130.5±2.1	41.0±0.0	248.0±2.8	171.5±2.1	76.5±0.7	7.6	64.2
CHRYSOSPORIUM TROPICUM GPCK 512							
5	302.0±2.8	30.5±0.7	351.0±1.5	332.5±2.1	18.5±3.5	8.1	74.9
10	312.0±1.4	32.5±3.5	367.5±2.1	344.5±4.9	23.0±7.0	8.0	84.8
15	342.0±1.4	37.0±1.4	449.0±1.4	379.0±2.8	70.0±7.0	7.3	86.7
20	184.5±0.7	39.0±1.4	337.5±2.1	223.5±2.1	114.0±2.1	8.2	77.2
25	134.0±1.4	41.0±0.0	222.5±2.1	175.0±1.4	47.5±0.7	7.4	55.1

When the experiment at the same water activity due to sucrose was conducted under shaking condition. The results were quite increasing as These strains showed superior ability to degrade the keratinous substrate. Thus C. tropicum GPCK 511 exhibited continuous increase in protein release of test sample, net protein release and weight loss up to 25 days of incubation recording peak values of 593.0 ug/ml, 204.0 ug/ml and 87.2 per cent (Table 5 and Fig. 1) in that order which illustrate superiority of shaking conditions over static condition.

C. tropicum GPCK 512 followed similar trend under shaking condition, however, results obtained showed lesser ability of this strain as compared to GPCK 511. The protein released in test sample maintained increasing values up to 25 days recording maximum of 469.0 ug/ml, same was the case with percentage weight loss (89.9%) at 25 days (Table 5).

TABLE 5: Keratinolytic Ability Of Two Different Strains Of Chrysosporium Tropicum At 0.95 aW Maintained By Using Sucrose 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	273.0±5.6	35.0±0.0	325.5±0.7	308.0±5.6	17.5±6.3	8.0	74.8
10	312.5±3.5	36.0±1.5	386.0±1.4	348.5±2.1	37.5±0.7	7.9	74.9
15	327.0±2.8	39.0±1.4	407.0±2.8	366.0±2.8	41.0±0.0	7.7	85.5
20	337.5±3.5	40.5±0.7	448.5 + 0.7	378.0±2.8	70.5±3.5	7.9	85.8
25	347.0±1.4	42.0±0.0	593.0±4.2	389.0±1.4	204.0±2.8	8.1	87.2
CHRYSOSPORIUM TROPICUM GPCK 512							
5	312.0±2.8	35.0±0.0	395.0±1.4	347.0±2.8	48.0±2.8	8.2	76.6
10	314.5±4.9	36.0±1.4	402.5±3.5	350.5±3.5	52.0±7.0	8.1	86.1
15	347.5±10.6	39.0±1.4	465.0±4.2	386.5±9.1	78.5±13.4	8.2	87.1
20	353.0±5.6	40.5±0.7	467.0±2.8	393.5±4.9	73.5±7.7	7.9	88.5
25	363.0±2.8	42.0±0.0	469.0±1.4	405.0±2.8	64.0±4.9	8.0	89.9

Increasing the concentration of sucrose to maintain the water activity at 0.93 seemed to decrease the keratinolytic ability of C. tropicum GPCK 511 and C. tropicum GPCK 512 as evidenced by results recorded in Table 6 for static condition. C. tropicum GPCK 511 continued to release protein from test sample in the increasing order up to 15 days of incubation thereafter it declined. However, there was sufficient loss of net protein release at 10 days of incubation showing negative value for the same. But the condition was considerably improved at 15 days with maximum net protein release (132.0 ug/ml). incubation period was increased to 20 and 25 days, a down fall in the trend was recorded. Weight loss was increased up to 62.1 per cent at 15 days which subsequently decreased to 60.3 and 59.0 per cent at 20 and 25 days respectively C. tropicum GPCK 512 under static condition released maximum protein in sample (3240) and 74.5 per cent weight loss at 10 days of incubation period.

Under shaking condition the overall results in respect of protein released in test sample net protein released weight loss by both the strains of C. tropicum were superior in nature (Table 7 and Fig. 3). C. tropicum GPCK 511 managed to release protein from test sample in increasing order up to 20 days of incubation thereafter it fell down to 299.5 ug/ml at 25 days. However, the trend was different in the case of net protein release, moreover, maximum value of 256.0 ug/ml were Achieved at 20 days of incubation. The weight loss due to degradation of human hair at 0.93 aW exhibited peak value of 90.0 per cent at 20 days of incubation.)

TABLE 6 : Keratinolytic Ability Of Two Different Strains Of Chrysosporium At Tropicum 0.93 aW maintained by using sucrose 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	66.5±0.7	11.0±1.2	121.0±1.4	77.5±2.1	43.5±0.7	7.7	51.5
10	131.5±0.7	14.0±0.0	137.5±0.7	145.5±0.7	-8.0	7.2	59.9
15	137.5±0.7	17.5±0.7	287.0±2.8	155.0±0.0	132.0±2.8	7.3	62.1
20	97.5±2.1	22.5±2.1	233.5±0.7	120.0±0.0	113.5±0.7	7.2	60.3
25	74.0±1.4	23.5±2.1	138.0±0.0	97.5±3.5	40.5±2.8	7.9	59.6
CHRYSOSPORIUM TROPICUM GPCK 512							
5	94.5±2.1	11.0±1.4	135.0±2.8	105.5±0.7	29.5±3.5	8.0	51.7
10	229.0±2.8	14.0±0.0	324.0±1.4	243.0±2.8	81.0±4.2	8.0	74.5
15	200.5±0.7	17.5±0.7	301.0±1.4	218.0±0.0	83.0±1.4	8.1	64.2
20	192.5±2.1	22.5±2.1	292.0±2.8	215.0±0.0	77.0±2.8	8.2	63.9
25	182.0±2.8	23.5±2.1	288.0±2.8	205.5±4.9	82.5±2.1	7.8	63.2

TABLE 7: Keratinolytic Ability Of Two Different Strains Of Chrysosporium Tropicum At 0.93 aW maintained by using sucrose 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	260.5±0.7	14.0±0.0	276.5±2.1	274.5±0.7	2.0±2.8	7.9	64.1
10	300.5±0.7	15.0±1.4	333.0±2.8	315.5±2.1	17.5±0.7	7.2	72.3
15	325.0±0.0	18.0±0.0	351.0±1.4	343.0±0.0	8.0±1.4	7.8	74.8
20	335.5±0.7	20.0±2.8	611.5±2.1	355.5±0.7	256.0±2.8	7.6	90.0
25	245.5±7.7	23.0±0.7	299.5±0.7	268.5±7.0	31.0±7.7	7.5	60.0
CHRYSOSPORIUM TROPICUM GPCK 512							
5	97.5±0.7	14.0±0.0	202.0±3.5	111.5±0.7	90.5±4.2	8.0	61.0
10	237.5±0.7	15.0±1.4	315.5±3.5	252.5±2.1	63.0±1.4	8.2	66.1
15	235.0±5.6	18.0±0.0	318.0±2.8	253.0±5.6	65.0±8.4	8.3	69.5
20	241.5±0.7	20.0±2.8	322.0±5.6	261.5±2.1	60.5±2.1	8.1	76.9
25	247.5±3.5	23.5±0.7	327.5±10.6	271.0±2.8	56.5±13.4	8.4	77.2

C. tropicum GPCK 512 showed continuous increase up to 25 days in respect of protein released in test sample (327.5 ug/ml) at 25 days. However, maximum net protein release was obtained at 5 days incubation period thereafter it showed tendency to decrease in the subsequent incubation period, whereas weight loss continued to increase (77.2%) up to 25 days (Table 7 and Fig. 4). Both the C. tropicum strains managed to behave in normal course even when the concentration of sucrose was further increased to 0.90 a as indicated by the results tabulated in Tables 8-9 and Figs. 4, under static and shaking conditions.

C. tropicum GPCK 511 showed continuous increase in protein release up to20 days of incubation thereafter it decreased. The net protein release also marked regular increase up to 20 days which came down to 80.5 ug/ml at 25 days, similar progressive trend was recorded in the case of weight loss 176 days.

However, the trend was different in the case of net protein release, moreover, maximum value of 256.0 ug/ml were achieved at 20 days of incubation. The weight loss due To degradation of human hair at 0.93 aW exhibited peak value of 90.0 per cent at 20 days of incubation.

C. tropicum GPCK 512 showed continuous increase up to 25 days in respect of protein released in test sample (327.5 ug/ml) at 25 days. However, maximum net protein release was obtained at 5 days incubation period thereafter it showed tendency to decrease in the subsequent incubation period, whereas weight loss continued to increase (77.2%) up to 25 days (Table 7 and Fig. 1). Both the C. tropicum strains managed to behave in normal course even when the concentration of sucrose was further increased to 0.90 a as indicated by the results tabulated in Tables 8-9 and Figs. 2, under static and shaking conditions.

C. tropicum GPCK 511 showed continuous increase in protein release up to 20 days of incubation thereafter it decreased. The net protein release also marked regular increase up to 20 days which came down to 80.5 ug/ml at 25 days, similar progressive trend was recorded in the case of weight loss up to 20 days (83.8%) which however, covered to 62.0 per cent at 25 days of incubation under static condition. C. tropicum GPCK 512 was comperatively less active under these conditions, however, it managed to show maximum value of 157.0 ug/ml, 34.5 ug/ml and 69.7 per cent at 15 days of incubation period for protein release in test sample, net protein released and weight loss respectively.

TABLE 8: Keratinolytic Ability Of Two Different Strains Of Chrysosporium Tropicum At 0.90 aW Maintained By Using Sucrose 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	121.0±1.4	14.0±5.6	135.0±4.2	12.5±2.1	147.5±2.1	6.9	62.0
10	133.0±0.0	15.0±4.2	228.0±1.4	148.0±4.8	80.0±2.8	7.0	65.7
15	135.5±0.7	19.0±0.0	248.0±1.4	154.5±0.7	93.5±2.1	7.1	66.8
20	54.5±2.1	20.0±1.4	440.5±9.0	74.5+3.5	366.0±2.1	7.3	83.8
25	36.0±1.4	21.0±0.0	137.5±3.5	57.0±1.4	80.5±2.1	7.4	62.0
CHRYSOSPORIUM TROPICUM GPCK 512							
5	38.0±4.2	14.0±5.6	68.5±0.7	52.0±9.8	16.5±10.6	6.8	51.0
10	99.5±4.9	15.0±4.2	145.0±4.2	114.5±0.7	30.5±4.9	7.6	61.4
15	103.5±3.5	19.0±0.0	157.0±2.8	122.5±3.5	34.5±6.3	8.0	69.7
20	54.0±4.2	20.0±1.4	97.0±2.8	74.0±2.8	23.0±0.0	7.9	59.5
25	21.5±2.1	21.0±0.0	39.5±0.7	42.5±9.1	-3.0	8.1	40.9

The keratinolytic ability of C. tropicum GPCK 511 at 0.90 aW under shaking condition is shown in Table 54 and Fig. 20. It revealed the liberation of higher amount of protein in test sample and net protein from the keratin substrate and also revealed higher weight loss (89.2%) of human hair. The protein release in test sample and net protein released showed the regular and increasing trend up to 20 days of incubation yielding 667.5 ug/ml and 422.0 ug/ml. The shaking condition at 0.90 aw also helped C. tropicum GPCK 512 favourably to degrade human hair and release protein. However, the extent of protein release in test sample and net protein release was lower than that of C. tropicum GPCK 511. Values of test sample's protein were maximum at 10 days incubation which then decreased at 15, 20 and 25 days of incubation period. The minimum value of net protein release was -26.0 at 25 days and maximum was 35.0 ug/ml at 10 days of incubation. The degradation of keratin substrate as indicated by weight loss was increased to 66.9 per cent at 20 days.

TABLE 9: Keratinolytic Ability Of Two Different Strains Of Chrysosporium Tropicum At 0.90 aW Maintained By Using Sucrose 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	274.0±1.4	18.0±0.0	295.0±0.0	292.0±1.4	3.0±1.4	8.0	75.4
10	332.5±3.5	19.5±0.7	376.5±2.1	352.0±2.8	24.5±4.9	8.1	84.9
15	337.5±3.5	21.5±2.1	395.5±4.9	359.0±1.4	36.5±6.3	8.4	86.9
20	222.0±2.8	23.5±0.7	667.5±2.1	245.5±3.5	422.0±1.4	8.5	89.2
25	202.5±3.5	25.0±1.4	357.5±3.5	227.5±4.9	130.0±8.4	8.2	77.1
CHRYSOSPORIUM TROPICUM GPCK 512							
5	63.0±0.0	18.0±0.0	98.0±1.4	81.0±0.0	17.0±1.4	8.2	51.5
10	97.5±0.7	19.5±0.7	152.0±5.6	117.0±1.4	35.0±7.0	8.1	71.7
15	113.0±1.4	21.5±2.1	143.5 2.1	134.5±3.5	9.0±0.7	8.2	61.6
20	118.5±1.4	23.5±0.7	134.0±6.3	142.0±1.4	-8.0	8.0	66.9
25	120.0±1.4	25.0±1.4	125.0±4.9	151.0±2.8	-26.0	8.3	65.0

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

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

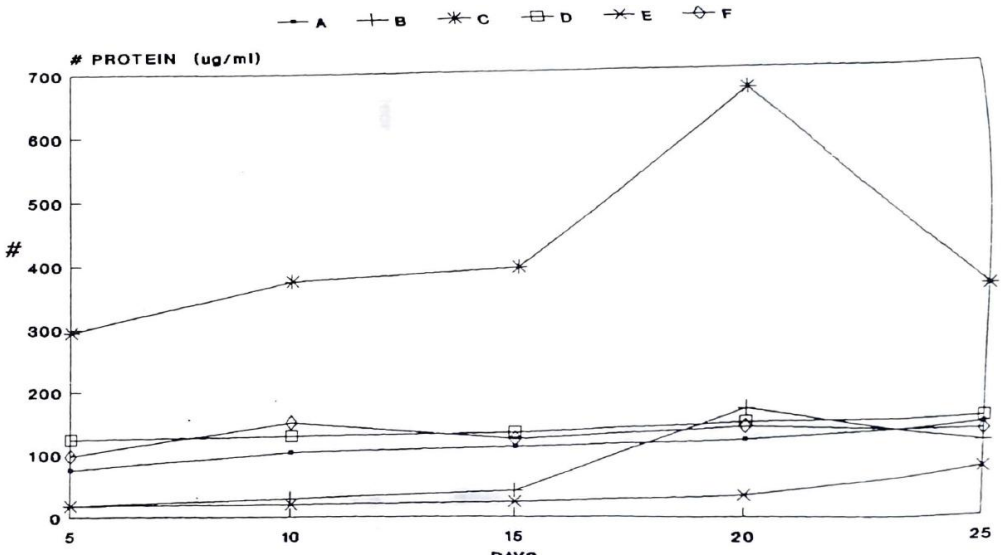

FIG. 1. Protein Released In The Culture Filtrate Of Chrysosporium Tropicum GPCK 511 AND Chrysosporium Tropicum GPCK 512 AT 0.90 a W controlled by KCl, NaCl and Sucrose in shaking condition using human hair.

The effect of 0.85 aw on the keratinolytic ability of C. tropicum GPCK 511 and C. tropicum GPCK 512 under static are given in Tables 1-2 and Figs1. and shaking conditions. The maximum protein released in test sample was recorded at 10 days of incubation, however, net protein released was 52.0 ug/ml at 5 days of incubation in case of C. tropicum GPCK 511. Weight loss was 62.6 at 5 days, 79.9 at 10 days, 72.0 at 15 days, 70.0 at 20 days and 69.5 per cent at 25 days of incubation period. C. tropicum GPCK 512 under static condition at the same showed maximum value of 278.5 ug/ml in 15 days incubation which decreased thereafter Net protein release was recorded higher in GPCK 512 than in GPCK 511 as it showed increasing trend up to 25 days of incubation period recording its maximum value of 70.5 ug/ml. The pH value after different incubatin periods centered around 8.0 to 8.3. The maximum weight loss was recorded 79.3 per cent at 15 days of incubation period.

Chrysosporium tropicum GPCK
A-KCI
B-NaCl
C-SUCROSE

Chrysosporium tropicum GPCK 512
D-KCI
E-N&CI
F-SUCROSE

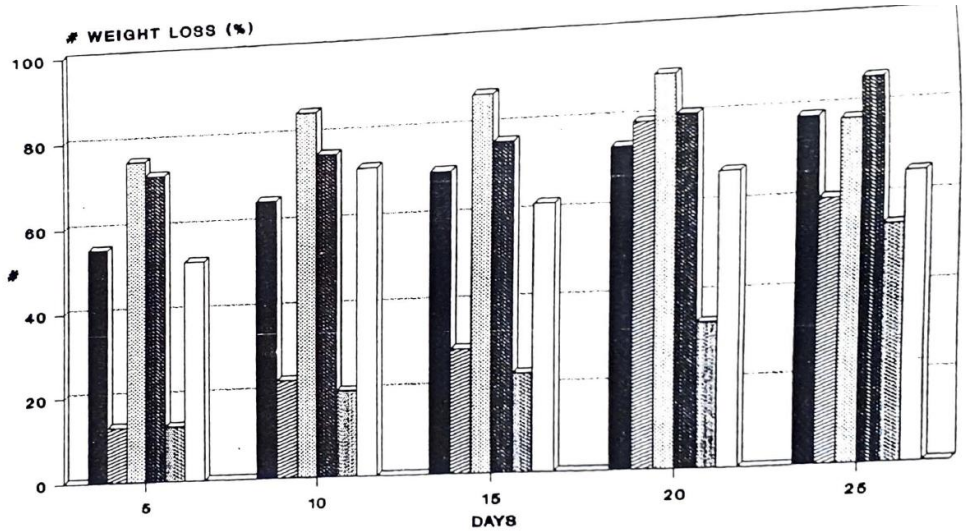

FIG. 2. Weight Loss Of Human Hair As Induced By Chrysosporium Tropicum GPCK 511 And Chrysosporium Tropicum GPCK512 AT 0.90 Aw Controlled By KC1, NaCl AND Sucrose In Shaking Condition.

TABLE 10 : Keratinolytic Ability Of Two Different Strains Of Chrysosporium Tropicum At 0.85 Aw Maintained By Using Sucrose 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	123.0±1.4	20.0±0.0	195.0±1.4	143.0±1.4	52.0±8.2	8.2	62.6
10	230.0±1.4	20.5±0.7	266.5±2.1	250.5±0.7	16.0±2.8	8.0	79.9
15	228.0±2.8	23.0±2.8	255.0±7.0	251.0±7.7	4.0±0.7	8.3	72.0
20	175.0±2.8	28.0±2.8	231.5±5.6	203.0±5.6	28.5±4.9	8.1	70.0
25	154.0±1.4	33.0±1.4	158.0±1.4	187.0±0.0	-29.0	8.2	69.5
CHRYSOSPORIUM TROPICUM GPCK 512							
5	177.0±8.4	20.0±0.0	215.5±6.3	197.0±8.4	18.5±14.8	8.0	70.0
10	214.0±18.3	20.5±0.7	250.0±2.8	234.5±17.6	15.52±0.5	8.1	72.7
15	226.5±6.3	23.0±2.8	278.5±2.1	249.5±3.5	29.0±5.6	8.0	79.3
20	177.5±3.5	28.0±2.8	247.5±3.5	205.5±6.3	42.0±2.8	8.1	72.0
25	124.0 + 5.6	33.0±1.4	227.5±3.5	157.0±4.2	70.5±0.7	8.2	71.0

The shaking condition at 0.85 aW considerably improved the keratinolytic ability of C. tropicum GPCK 511 and C. tropicum GPCK 512 as shown in Table 10. The protein released in test sample and net protein release showed regular increasing trend up to 20 days of incubation thereafter it decreased at 25 days. The best values of protein release in test sample and net protein release were recorded to be 672.5 ug/ml and 296.5 ug/ml respectively. The percentage weight loss reached up to 98.2 per cent at 20 days of incubation. The magnitude of protein release in test sample and net protein released was somewhat inferior in the case of C. tropicum GPCK 512 than C. tropicum GPCK 511. Under shaking condition at 0.85 aw the strain managed to release protein from test sample to the extend of 276.0 ug/ml at 15 days whereas maximum 53.5 ug/ml net protein release was at 20 days of incubation period. The weight loss was found to maximum value of 74.8 per cent at 15 days of incubation.

The study conducted on the effect of different water activities from 0.98 to 0.85 i.e. under water stress in the of three different solutes (KC1, NaCl and sucrose) presence revealed wide variations in respect of protein in test sample, net protein release and weight loss indicating the impact of water stress on the biodegradation of human hair.

TABLE 11: Keratinolytic Ability Of Two Different Strains Of Chrysosporium Tropicum AT 0.85 aW Maintained By Using Sucrose 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	279.5±0.7	25.0±0.0	315.0±7.0	304.5±0.7	10.5±7.7	7.9	80.9

10	337.5±3.5	25.5±2.1	390.5±0.7	363.0±1.4	27.5±2.1	7.6	86.5
15	342.5±3.5	28.0±1.4	399.5±0.7	370.0±2.1	29.0±2.8	8.0	88.0
20	347.5±10.6	28.5±2.1	672.5±3.5	376.0±8.4	296.5±12.0	7.9	98.2
25	196.0±2.8	31.0±1.4	357.5±3.5	227.0±1.4	130.5±2.1	7.8	73.3
CHRYSOSPORIUM TROPICUM GPCK 512							
5	179.0±5.6	25.0±0.0	210.5±0.7	204.0±5.6	6.5±4.9	8.0	63.1
10	218.5±12.0	25.5±6.3	253.5±6.3	244.0±9.8	9.5±3.5	8.1	73.9
15	228.0±4.2	28.0±1.4	276.0 ±3.5	256.0±2.8	20.0±1.4	7.9	74.3
20	172.5±10.6	28.5±2.1	254.5±6.3	201.0±8.4	53.5±14.8	7.5	70.2
25	128.5±0.7	31.0±1.4	232.5±3.5	159.5±2.1	73.0±1.4	8.2	69.9

Chrysosporium tropicum GPCK 511
A-KCI
B-NaCl
C-8UCROSE

Chrysosporium tropicum GPCK 612
D-KCI
E-N CI
F-SUCROSE

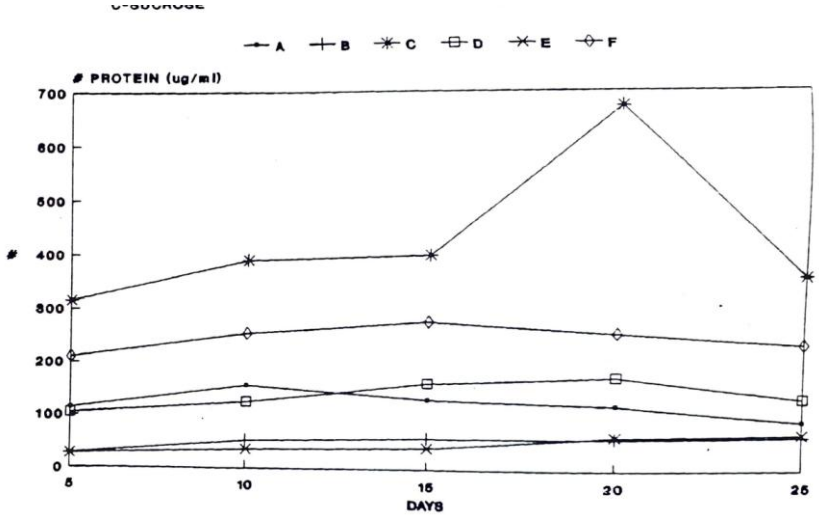

FIG. 3. Protein Released In The Culture Filtrate Of Chrysosporium Tropicum GPCK 511 And Chrysosporium Tropicum GPCK 512 at 0.85 aW controlled by KCI, NaCl and Sucrose In Shaking Condition Using Human Hair.

In most of the cases it was found that among the three solutes used, sucrose favoured increase in the value of protein in test sample and net protein release and percentage weight loss. With the exception in some cases the efficiency of biodegradation by C. tropicum GPCK 511 was superior in shaking condition as compared to static condition, for example, if the keratinolytic ability of C. tropicum GPCK 511 and GPCK 512 at the water activity of 0.98 aWare compared, strain GPCK 512 was found to be over all superior when sucrose was used as solute than that of KC1 and NaCl. Similar trend on the protein in test sample, net protein and percentage weight loss was recorded at the a 0.95 indicating that presence of added sucrose favoured more protein release, net protein value and weight loss by both the strains under static and shaking conditions. Again the keratinolytic ability of strain GPCK 512 was noticed to be better at this water level.

Decreasing the water availability to 0.93aW i.e. increasing the concentration of solutes which created water stress resulted into decrease in the value of protein release in test sample, net protein and weight loss in the case of both the C. tropicum strains.

Chrysosporium troploum GPCK 511
A-KCI
B-NaCI
C-SUCROSE

Chrysospirium trpicum GP CK 512
D-KCI
E-NaCI
F-SUCROSE

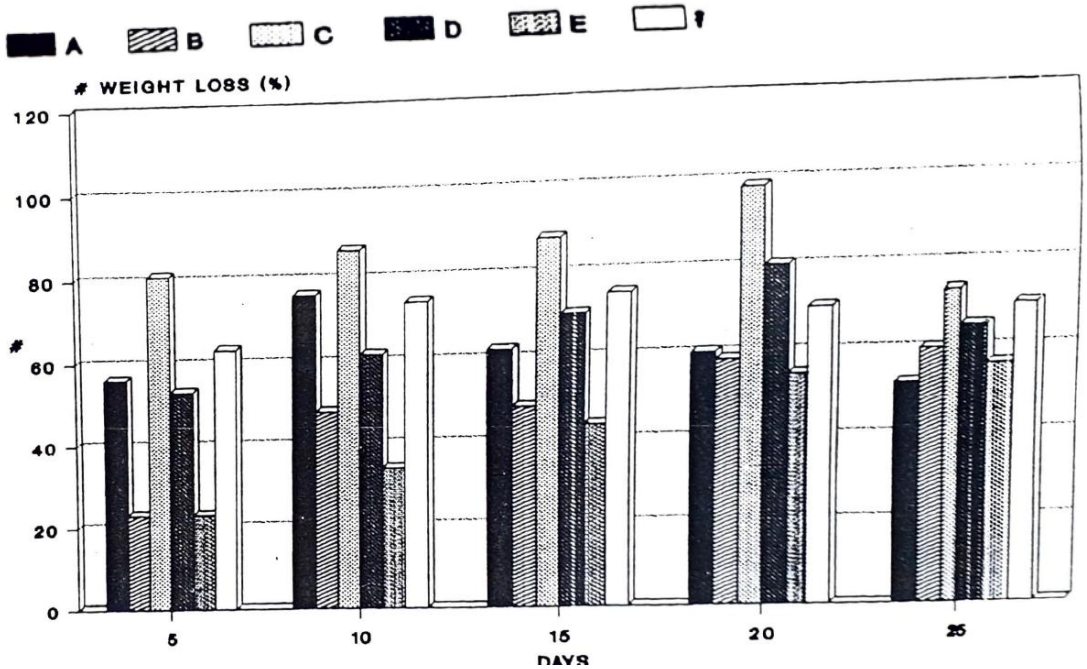

FIG. 4. Weight Loss Of Human Hair As Indused By Chrysosporium Tropicum GPCK 511 And Chrysosporium Tropicum GPCK 512 at 0.85 aw Controlled By KC1, NaCl AND Sucrose In Shaking Condition.

It was noted that in water stress (0.90 aw) increased protein of test sample, net protein and weight loss in the case of strain GPCK 511 as compared to the results obtained at aW 0.93 However, the keratinolytic ability of strain GPCK 512 showed lower values for protein in test sample, net protein and weight loss at the aW 0.90 as compared to 0.93aw'

When the results of protein in test sample, net protein and weight loss at 0.85 aW in case of strain GPCK 511 were compared to the results obtained at the 0.90 aW there was considerable decrease in protein value of test sample, net protein and weight loss under static condition, however, under shaking condition values for protein in test sample, net protein and weight loss showed further increase. C. tropicum GPCK 512 was not that much susceptible to water stress at the 0.85 a and showed increasing trend in respect of protein of test sample, net protein and weight loss as compared to the results obtained at the 0.90 a

The results indicated that in general, there appeared to be no correlation between levels of water stress and degradation ability of C. tropicum GPCK 511 and GPCK 512 as optimum activity of these strains appear to be governed by various variables used in the experiment, for example incubation periods, type and amount of the solute used to create water stress, reaction conditions employed (static and shaking) and the C. tropicum strains used.

As for example the keratinolytic ability of C. tropicum GPCK 511 under static condition was recorded to be maximum in respect of protein release in test sample (440.0 ug/ml), net protein release (366.0 ug/ml) and weight loss (83.0%) at 20 days of incubation period in a, 0.90 when sucrose was used as a controlling solute whereas in shaking condition the same strain demonstrated its maximum capability to degrade human hair showing protein of test sample, net protein and weight loss to the extent of 667.5 ug/ml, 4220 ug/ml and 89.2 per cent respectively at 20 days of incubation period at a 0.93 aW containing sucrose as solute.

Similarly C. tropicum GPCK 512 showed optimum values for protein in test sample (449.0 ug/ml) at 15 days of incubation, net protein of 114.0 ug/ml at 20 days of incubation period and weight loss (86.7%) at 15 days of incubation period at the water activity of 0.95 in the presence of prescribed amount of sucrose. However, under shaking condition the same strain exhibited optimum values for protein release in test sample (469.0 ug/ml) at 25 days of incubation, net protein release (78.5 ug/ml) at 15 days of incubation period and weight loss (89.9%) at 25 days of incubation period at the same aW in the presence of sucrose. It may be recorded that values for net protein were superior in the static conditions as compare to shaking condition.

Water stress condition induced by adding different levels of potassium chloride, sodium chloride and sucrose also had negative impact on the net protein released from human hair by the keratinolytic activity of C. tropicum GPCK 511 and GPCK 512. The negative impact on the net protein released could be observed mostly due to the effect of sodium chloride addition at 0.95 aW and 0.85 aW under static condition when GPCK 511 was used as keratinolytic agent. Similarly in case of GPCK 512 also negative values for net protein released at

different incubation periods under the influence of sodium chloride in static condition at the a of 0.98, 0.95 and 0.85 were recorded under shaking condition it was recorded at aw of 0.93 and 0.90. However, negative impact of potassium chloride at the water activity of 0.90 under shaking condition could be recorded at 5, 10 and 15 days of incubation period only in case of C. tropicum GPCK 511. While at most of the levels of water activities sucrose favoured high values of protein released in case of both the strains, however, it also showed negative impact when concentration of sucrose was maintained at the water activity of 0.93 under static condition in the case of C. tropicum GPCK 511. The adverse effect of sucrose addition could also be seen in the case of C. tropicum GPCK 512 when the water activity was maintained at 0.90 under static and shaking conditions both. It appears that increasing the levels of solute in the mineral media increase the Values of net protein released up to a specific optimum concentration thereafter it decrease when the concentration of sucrose is too high. Moreover, shaking condition employed in the experiments in general played crucial role in favouring high increase in the keratinolytic ability of C. tropicum GPCK 511 and GPCK 512.

The supporting role of controlling solute at different water activities towards fungal growth has also been discribed by Saxena (1993). Magan and Lacey (1948b) while studying water relation of same plant pathogenic fungi found that optimum and minimum values for water activity for different species of fungi varies from species to species and requires specific optimum temperature for optimum fungal growth. In addition to the above report Cuero et al., (1987); Wheeler et al., (1988a); Griffin (1972) and Wearing and Burgess, (1979), also reported the effect of water activities on the growth of several strains of fungi, some of which are in accordance with the results reported in this chapter.

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