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Isolation and Optimization of Protease Producing Thermophilic Fungal Strains from Warangal

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ABSTRACT:- The thermophilic fungi were positive for the production of protease T. lanuginosus, R.pusillus, R.miehei, H.grisea, H.insolens and A.fumigatus produced maximum amount of protease, while T. aurantiacus, C. fergusii, M. pulchella, T. luteus were responsible for low protease activity. Production of protease, mycelial growth and pH changes in different synthetic media by three strains of T.lanuginosus is mentioned in the table:1-6 and evaluated graphically Fig 16-20. Protease secreted by all the three strains in albumin containing medium was make much more difference on the yield of mycelial growth. A positive correlation could be drawn between the specific activity and protease secreted by fungi. Mycelial growth of GSLMBKU-14 and GSLMBKU-12 was more in medium F, while medium B induced same in GSLMBKU-10. Medium E was inferior in induction of mycelial growth of GSLMBKU-12, while with the other two strains medium C was responsible for least mycelial growth. Supplementation of albumin to medium A, C, and E did not make much difference on the yield of mycelial growth. Medium F was the best for the secretion of protease by GSLMBKU-14, while medium B induced maximum protease production in GSLMBKU-14 and GSLMBKU-10. Medium A was inferior to medium D for production of proteases by GSLMBKU-12. Addition of albumin to medium C has no significant change in protease production by all the three strains.

***KEY WORDS*:** Protease, Thermophilic fungi, Mycelial, *GSLMBKU* strain.

INTRODUCTION

Proteases are one of the most important groups of derivative enzymes that catalyze the hydrolysis of protein by attacking the peptide bond which links amino acids. Proteases represent one of the three largest groups of industrially important enzymes[1-4]. They are commercially available and are widely distributed in nature i.e; found in all living organisms .In humans; they are prevalent in many physiological functions, both normal and disease-related. But when compared to proteases obtained from plant and animal, microbial proteases are preferred mainly because of short generation time, the ease of bulk production, genetic manipulation and extra cellular secretion which simplifies the downstream processing of the enzyme [5-7]. Nearly all major groups of microorganisms including bacteria, fungi and actinomycetes are known to produce proteases intracellularly and extracellularly[8] exhibiting exopeptidase and endopeptidase activities [9-10].

Fungal proteases are mainly used in food, Dairy, Textile, Detergent industries and more recently, in the leather industry for dehairing, bating and tanning hides. They are also used in certain other sectors like diagnostics, medicine and surgery, animal cell culture and molecular biology.

MATERIALS AND METHOD

An extensive and intensive survey of different ecological niches such as coal mine area, municipal dump yard, bird nests etc were made in and around Warangal of Telangana region with luxuriant vegetation and varied climate of extreme temperatures and rainfall. Average temperature ranges from 25˚C to 48˚C through major part of the year, where as rainfall is less than 18 mm and region is dry with less humidity which leads to maximum biodiversity. Further, it is the main mining area of Andhra Pradesh. Coal mines associated with other industries like paper mills, power plants, Rayan factory, Beedi factories and tanning industries are located in this area. Coal mine area with open cast mining covering about 100 km and the zoo situated in Warangal was selected for the present studies. Besides these habitats, mushroom compost, decomposing plant material, municipal wastes, wood chip piles, cattle dung, vermicompost, zoo waste, poultry waste and furnace soil etc were surveyed for the presence of thermophilic fungi.

The present fungal strains exhibited greater specificity towards nitrogen source present in the medium for both mycelial growth and protease production. *GSLMBKU-14* and *GSLMBKU-12* could produce maximum protease in medium containing L-asparagine, followed by yeast extract. El-Gindy *et al.* (2008) reported that *A. awamori* produced good amount of protease in yeast extract (0.05, 0.10, 0.15, 0.20, 0.25%). Protease production was higher when optimized medium was fortified by (0.20%)

yeast extract for *A. awamori*. The protease production was very low in medium containing ammonium chloride and L-histidine. Twelve days incubation period was optimum for mycelial growth and protease production by all the three strains under investigation. *GSLMBKU-10* preferred yeast extract for the maximum production of protease. Yoon *et al.* (2009) also reported that *A.niger* could produce good amount of protease in medium containing yeast extract. Asparagine and L-arginine were next preferred substrates. L-histidine, methionine and ammonium sulphate were poor substrates for the production of protease by *GSLMBKU-10.*

GSLMBKU-10 produced maximum amount of protease in medium containing galactose carbon source and equal amount of protease on starch and D-fructose. Maltose followed by mannitol was the next preferred carbon sources for the production of protease by *GSLMBKU-10,* while Citric acid, succinic acid, D-ribose and D-xylose were poor carbon sources for the production [11-12] of protease has also reported significant influence of carbon source on protease production by fungi studied by them. Mannose supported maximum production of protease by *GSLMBKU-12,* while mannitol, D-galactose, fructose, sucrose and starch were next preferred substrates.

RESULTS AND DISCUSSION

Table 1. Protease production (in mg/ml) by different thermophilic fungi

Name of the fungus	Incubation Period (in days)	pH	Dry weight (mg/ml)	Protease (in IU ml-1)
Chaetomium thermophile. V caprophile	6	6.2	135.0	0.06
	9	6.5	186.0	0.08
	12	6.8	213.0	0.07
Chrysosporium fergusii	6	6.0	152.0	0.05
	9	6.6	203.0	0.09
	12	6.9	227.0	0.08
H.grisea	6	6.3	184.0	0.08
	9	6.8	223.0	0.1
	12	7.0	241.0	0.07
Humicola insolens	6	5.6	158.0	0.07
	9	6.1	193.0	0.09
	12	6.5	216.0	0.06
Rhizomucor miehei	6	6.3	163	0.06
	9	6.7	182.0	0.1
	12	6.9	197.0	0.08
R. pusillus	6	6.0	178.0	0.08
	9	6.4	219.0	0.11
	12	6.7	235.0	0.07
Malbranchea pulchella	6	6.5	138.0	0.03
	9	6.9	168.0	0.06
	12	7.1	185.0	0.05
Talaromyces luteus	6	6.2	128.0	0.02
	9	6.6	171.0	0.05
	12	6.9	193.0	0.04

Thermoscus aurantiacus	6	6.0	143.0	0.05
	9	6.4	167.0	0.09
	12	7.0	187.0	0.07
T. lanuginosus GSLMBKU-10	6	6.1	184.0	0.09
	9	6.5	214.0	0.14
	12	6.6	231.0	0.08
T. lanuginosus GSLMBKU-11	6	6.4	166.0	0.07
	9	6.8	198.0	0.09
	12	7.1	208.0	0.07
T. lanuginosus GSLMBKU-12	6	6.2	179.0	0.08
	9	7.1	201.0	0.11
	12	6.7	243.0	0.09
T. lanuginosus GSLMBKU-13	6	6.6	159.0	0.08
	9	6,7	185.0	0.1
	12	7.2	197.0	0.08
T. lanuginosus GSLMBKU-14	6	6.3	192.0	0.09
	9	6.6	237.0	0.13
	12	6.8	257.0	0.07
Torula thermophila	6	6.1	127.0	0.04
	9	6.5	154.0	0.08
	12	7.2	176.0	0.07

Table 2. ANOVA of Lipase production on different synthetic media by three strains of *T.lanuginosus*

Sources of variation	Sum of Squares	df	MS	F	P	Result
Between groups Within Groups Total	818.9259 1273.2593 2445.2593	2 17 53	409.463	39.43	<.0001	S

S- Significant

Table 3. ANOVA of Effect of pH on Lipase production by three strains of *T.lanuginosus*

Sources of variation	Sum of Squares	df	MS	F	P	Result
Between groups Within Groups Total	206.5714 1574.7619 2225.4286	2 20 62	103.2857	9.3	0.000482	S

S- Significant

Table 4. ANOVA of Effect of Temperature on Lipase production by three strains of *T.lanuginosus*

Sources of variation	Sum of Squares	df	MS	F	P	Result
Between groups Within Groups Total	504.1333 3587.3333 4708	2 14 44	252.0667	11.45	0.000232	S

S- Significant

Table 5. ANOVA of Influence of different carbon sources on Lipase production by three strains of *T.lanuginosus*

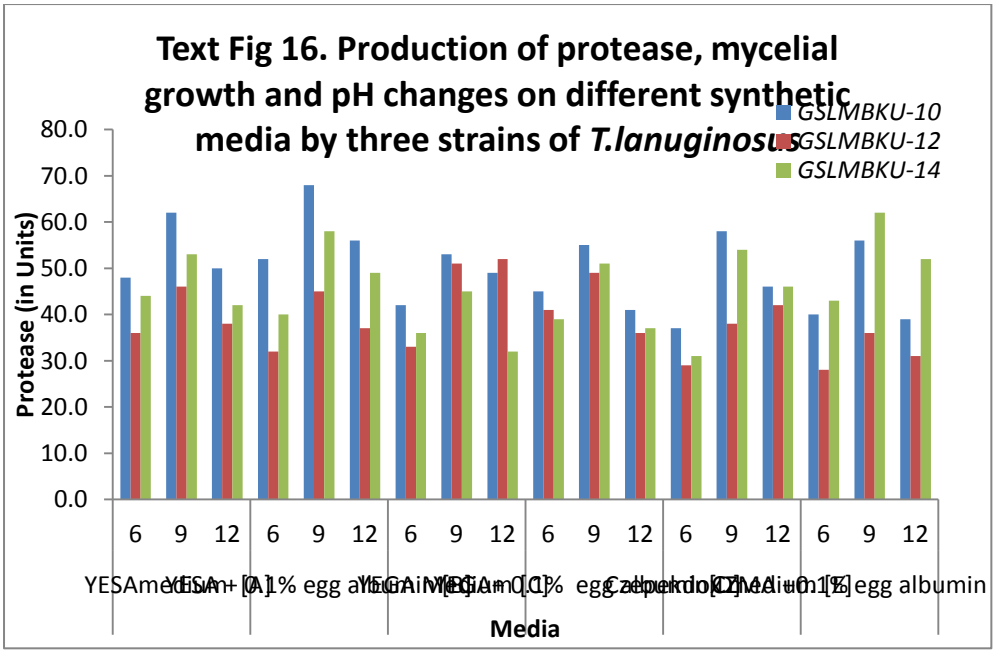
Sources of variation	Sum of Squares	df	MS	F	P	Result
Between groups Within Groups Total	62.5926 5221.9259 6138.5926	2 44 134	31.2963	3.22	0.044707	S

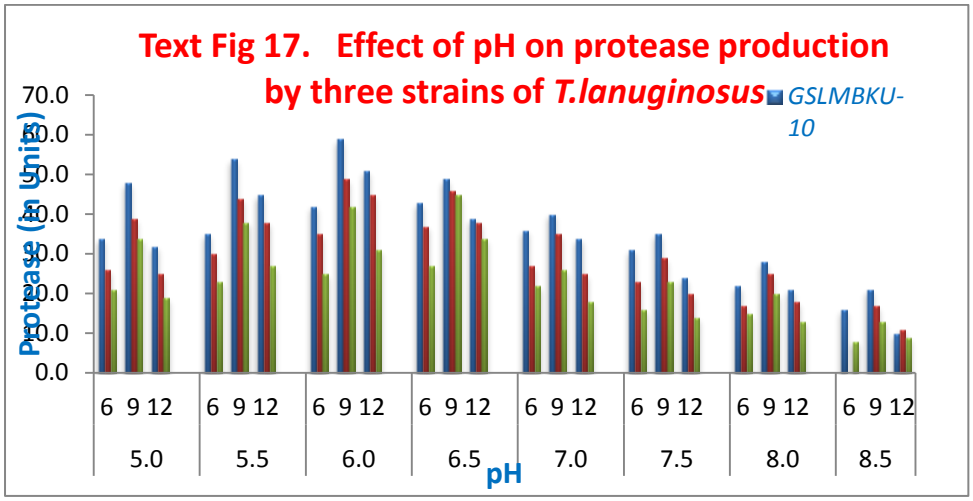
S- Significant

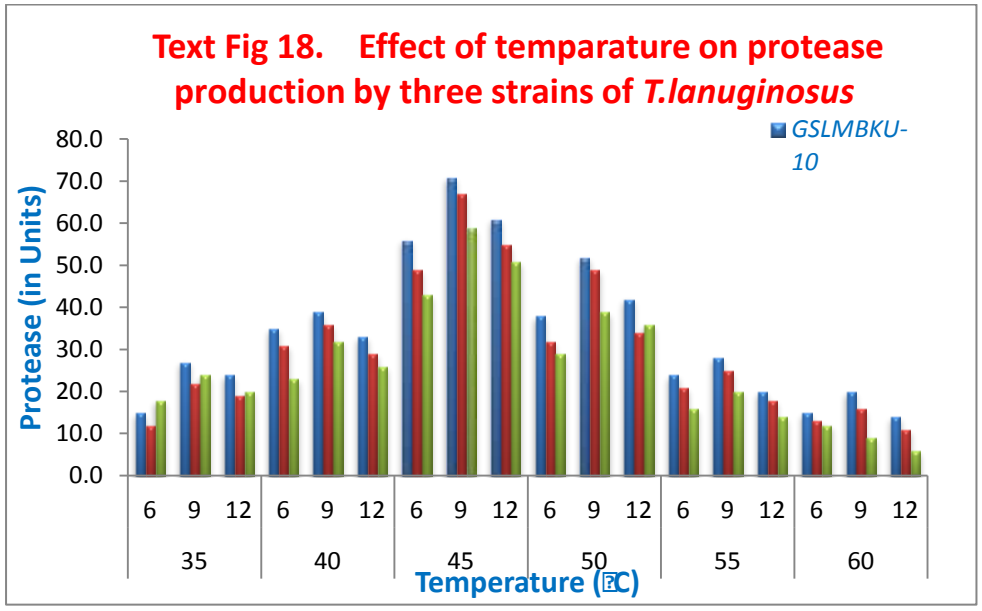
Table 6. ANOVA of Influence of different Nitrogen sources on Lipase production by three strains of *T.lanuginosus*

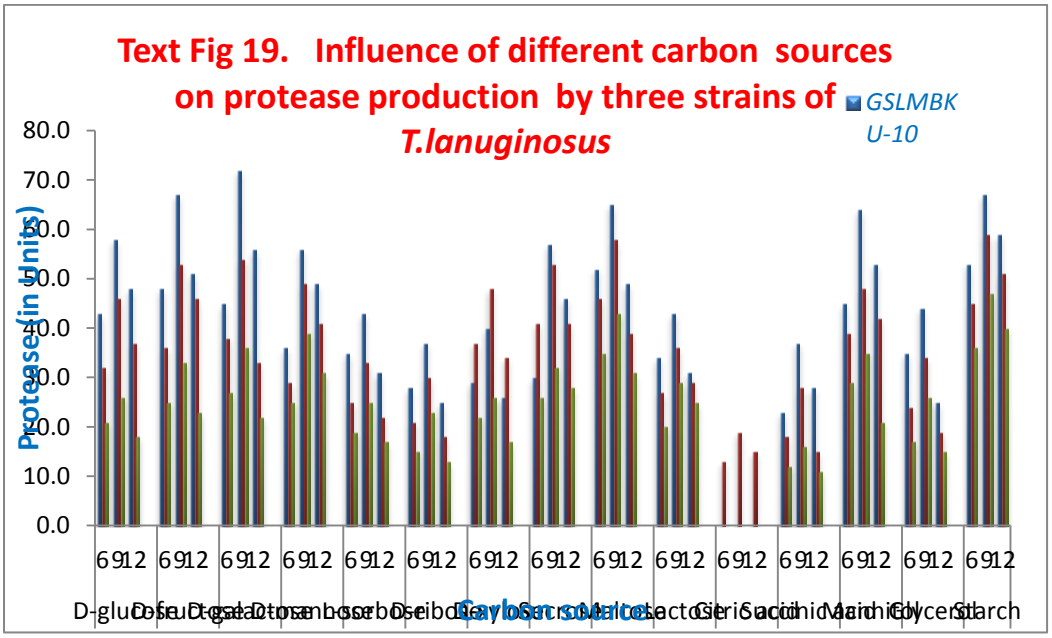
Sources of variation	Sum of Squares	df	MS	F	P	Result
Between groups Within Groups Total	1312.0392 13580.4314 19237.7647	2 50 152	656.0196	15.1	<.0001	S

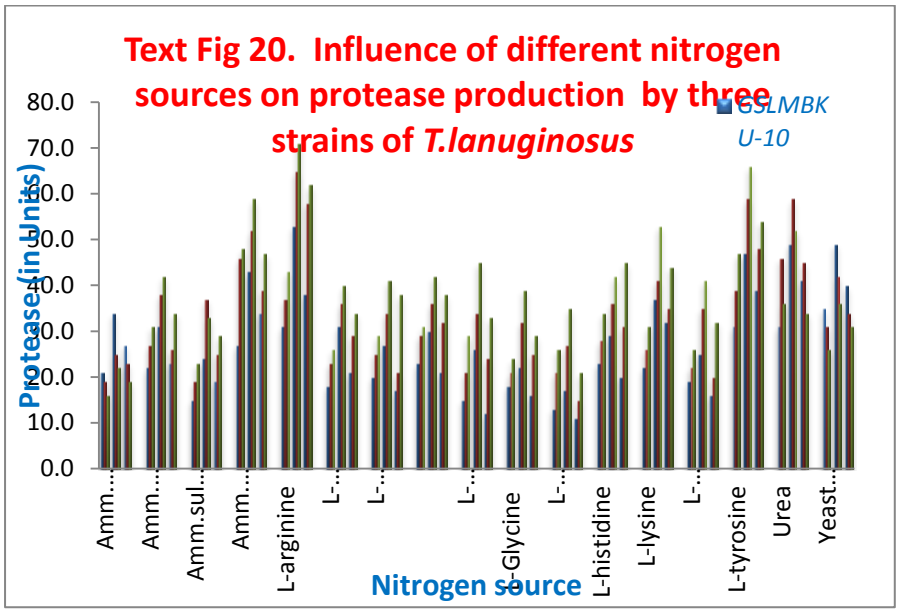
S- Significant




Text Fig 17. Effect of pH on protease production by three strains of T.lanuginosus
GSLMBKU-10
Protease (in Units)
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
6 9 12
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
pH


Text Fig 18. Effect of temparature on protease production by three strains of T.lanuginosus
GSLMBKU-10
Protease (in Units)
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
6 9 12
35
40
45
50
55
60
Temperature (°C)





CONCLUSION

GSLMBKU-10 could accomplish good mycelial growth in all the nitrogen sources tried. A positive correlation could be observed between mycelial growth and protease production. All are displayed in tabulation and represented by graphically.

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