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Screening and Culture Conditions for the Production of Amylase by PH, ANOVA by using GSLMBKU Fungal Strains

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ABSTRACT:- Herewith discuss the *T.luteus* and *T. lanuginosus* were showed clear zones when flooded with iodine solution, suggesting their potential to secrete amylase, while remaining fungi were failed to produce amylase. α-amylase production by all the three strains of *T. lanuginosus*, while medium E was poor substratum for α-amylase production. Medium F was more suitable for β-amylase production by *GSLMBKU* strains.Conditions are optimized by maintaing PH. *GSLMBKU-10* could achieve good mycelial growth during its growth on L-asparagine and yeast extract, while it was least in medium containing L-glutamic acid. Glycine was more suitable nitrogen source for the vegetative growth of *GSLMBKU-12*, whereas ammonium sulphate was least preferred. Further confirmed by ANOVA studies.

KEYWORDS

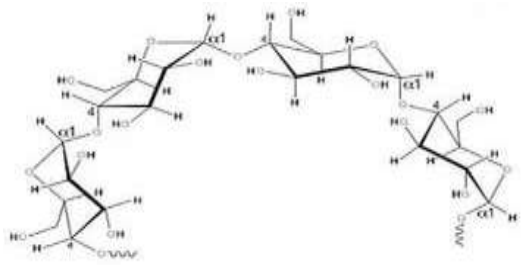
Amylases, amylopectin, ANOVA,fungal strain.

INTRODUCTION

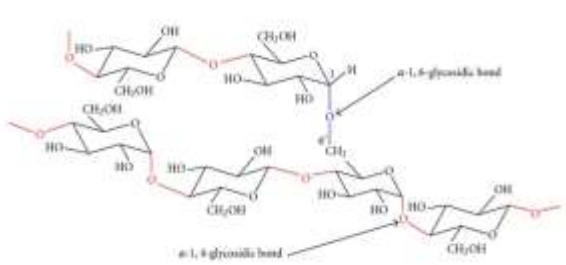
Amylases are important hydrolytic enzymes which catalyzes the hydrolysis of polysaccharides such as starch into simple sugars[1]. The major industrial sources of starch are the grains of cereals (maize, wheat, rice) and tubers (potato and cassava). Starch is a polymer of glucose linked to one another through the glycosidic bonds[2-4]. Starch is composed of two distinct polysaccharides amylose and amylopectin. Amylose is essentially a linear polymer consisting of up to 6000 glucose units with α(1-4) glycosidic bonds .The average amylose content in most common starches [5-6] such as barley, corn and potato, is 20-30%. Amylopectin is a highly branched polymer consisting of short α, 1-4 linked linear chains of 10–60 glucose units and α,1-6 linked side chains with 15–45 glucose units. Altogether the complete amylopectin molecule contains about 2 000,000 glucose units, thereby being one of the largest molecules in nature. Internal part of amylopectin is considered to be critical to the physical behavior of granular starch.

Amylases are group of enzymes which are capable of digesting these glycosidic linkages found in starches. These enzymes are used in starch processing industries to produce ethanol, glucose and fructose syrups and they are also used in textile, brewing and paper industries[7-8]. Amylases are widely distributed in plants, animals and microorganisms which differ widely in their properties and mode of action depending on the source. In spite of the wide distribution, the microbial amylases are preferred over plant and animal source for industrial production. This is because of several advantages such as cost effectiveness, consistency, less time and space required for bulk production as well as ease of process modification and optimization. Amylases produced by thermophilic fungi are especially more important because of thermo stable enzymes, which can be used in saccharification processes that occur at high temperature [9-10].

Structure of amylose



Structure of amylopectin



MATERIALS AND METHOD

Amylase production by three strains of *T. lanuginosus* on different synthetic media was studied. Simultaneously vegetative growth and pH changes were also recorded and the results are summarized in **Table 1, 2**.Name of the fungus and ANOVA studies mentioned from **Table 3-13**.

α-amylase production by all the three strains of *T. lanuginosus*, while medium E was poor substratum for α-amylase production. Medium F was more suitable for β-amylase production by *GSLMBKU-10*. Whereas, in *GSLMBKU-12* and *GSLMBKU-14* medium B was responsible for maximum β-amylase production. Addition of starch to media was not greatly influenced enzyme production by *GSLMBKU-10,* where as in other two strains, enzyme production influenced by addition of substrate. Nine days incubation was optimum for the enzyme production. Medium D followed by medium C were best substrates for the growth of *GSLMBKU-10*, while *GSLMBKU-12* opted medium B followed by C and *GSLMBKU-14* opted medium B followed by D. Nine days incubation period was optimum for the vegetative growth in all the media tried. Minimum pH changes were recorded and the final pH remained near neutral. The pH range of 5.0 to 8.0. Maximum α-amylase production observed at pH 6.5, whereas pH 6.0 was optimum for β amylase production.. *GSLMBKU-12* could grow in the pH range of 5.0 to 7.5. It failed to grow at pH 8.0. pH 6.0 followed by pH 6.5 was most suitable for both α and β amylase production. *GSLMBKU-14* was able to grow in the pH range of 5.0 to 8.0 and pH 6.5 followed by pH 7.0 was optimum for α-amylase production and pH 6.0 for β amylase production of range pH 6.0 to 7.0 was optimum for all the three strains under investigation.**(Text Fig 12,13)**

RESULTS AND DISCUSSION

Table 1. Production of amylase*, mycelial growth and pH changes on different synthetic media by three strains of *T.lanuginosus*

		GSLMBKU-10				*GSLMBKU-12*				*GSLMBKU-14*			
Medium	Days of incubation	Dry wt (µg/ml)	pH	Amylase (in units) α	Amylase (in units) β	Dry wt (µg/ml)	pH	Amylase (in units) α	Amylase (in units) β	Dry wt (µg/ml)	pH	Amylase (in units) α	Amylase (in units) β
Yeast extract Starch	6	176.0	5.9	124.0	194.0	158.0	5.5	101.0	91	142.0	6.0	121.0	82.0
medium [A]	9	192.0	6.3	167.0	119.0	174.0	6.4	139.0	112	165.0	6.4	154.0	95.0
	12	217.0	6.8	135.0	87.0	186.0	6.7	97.0	85	181.0	6.8	118.0	73.0
Medium A +	6	184.0	6.2	157.0	158	179.0	5.8	126.0	109	155.0	5.6	147.0	91.0
0.1% starch [B]	9	205.0	6.8	211.0	229	196.0	6.6	166.0	138	178.0	6.2	198.0	112.0
	12	229.0	7.2	185.0	176	213.0	6.9	112.0	124	202.0	7.1	174.0	86.0
Yeast extract Glucose	6	197.0	5.7	116.0	108	167.0	6.4	82.0	72.0	135.0	6.1	135.0	64.0
Medium[C]	9	221.0	6.4	158.0	129	185.0	6.9	95.0	97.0	169.0	6.7	169.0	78.0
	12	243.0	6.9	134.0	112	196.0	7.0	63.0	52.0	183.0	6.8	127.0	52.0
Medium [C]+	6	209.0	6.1	137.0	131	159.0	6.1	91.0	79.0	147.0	6.3	151.0	69.0
0.1% starch [D]	9	225.0	6.9	173.0	168	172.0	6.7	115.0	106.0	188.0	6.5	208.0	76.0

	12	247.0	7.4	114.0	110	189.0	7.3	86.0	88.0	195.0	7.0	182.0	55.0
Modified Czepek dox medium[E]	6	164.0	6.7	96.0	146	131.0	6.5	67.0	81.0	125.0	7.1	114.0	71.0
	9	178.0	7.1	123.0	201	143.0	6.9	83.0	93.0	136.0	7.3	125.0	98.0
	12	191.0	7.5	67.0	183	155.0	7.2	45.0	76.0	151.0	6.6	107.0	65.0
Medium [E] + 0.1 % starch [F]	6	174.0	6.3	98.0	153	139.0	6.2	72.0	92.0	142.0	6.5	123.0	84.0
	9	188.0	6.6	129.0	211	169.0	7.0	89.0	116.0	158.0	7.2	144.0	102.0
	12	211.0	7.6	77.0	191	161.0	7.4	54.0	63.0	160.0	7.8	132.0	63.0

*Expressed in units, one unit of amylase is that amount of protein which will hydrolyse ten mg of starch per minute under specific conditions

Table 2. Effect of temperature on amylase* production, mycelial growth and pH changes by three strains of *T.lanuginosus*

		GSLMBKU-10						*GSLMBKU-12*		*GSLMBKU-14*			
Temp- Armature	Days of incubation	Dry wt (µg/ml)	pH	Amylase (in units)	Amylase (in units)	Dry wt (µg/ml)	pH	Amylase (in units)	Amylase (in units)	Dry wt (µg/ml)	pH	Amylase (in units)	Amylase (in units)
				α	β			α	β			α	β
35	6	--	--	--	--	67	5.5	21	43	--	--	--	--
	9	84	5.4	45	63	78	5.9	32	59	--	--	--	--
	12	112	5.9	37	46	102	6.3	25	31	--	--	--	--
40	6	142	6.1	75	103	114	5.8	52	86	119	6.3	64	96
	9	169	6.4	124	164	135	6.2	93	128	123	6.5	82	137
	12	186	6.8	86	123	121	6.7	68	92	139	7	51	110
45	6	174	6.5	106	161	156	5.8	129	135	142	6.6	113	143
	9	236	6.7	158	226	195	6.4	165	194	173	7	134	215
	12	251	7.1	117	187	227	7.2	115	174	192	7.2	108	179
50	6	196	6.3	147	134	133	5.5	85	126	127	6.4	73	121
	9	257	6.8	186	207	148	6.9	98	188	153	6.8	91	156
	12	218	7.4	206	178	151	7.4	69	157	169	7.5	43	136

55	6	127	6.6	68	97	101	6.5	49	76	89	7.2	36	67
	9	145	7.2	81	104	116	6.8	62	92	106	7.5	73	85
	12	158	7.6	42	65	112	7.5	35	48	95	7.6	39	52

*Expressed in units, one unit of amylase is that amount of protein which will hydrolyse ten mg of starch per minute under specific conditions

Table 3. Production of amylase by different thermophilic fungi

Name of the Fungus	Zone of clearance
Absidia corymbifera	--
Acremonium thermophilum	--
Aspergillus fumigatus	+
A. nidulans	+
A. terreus	+
A. flavus	+
A.niger	+
Chaetomium thermophile. V caprophile	+
Chrysosporium fergusii	--
Humicola grisea	+
H.fuscoatra	--
Humicola insolens	+
H.stellata	+
Rhizomucor miehei	+
R. pusillus	+
Rhizopus arrhizus	+
R.microsporus	+
Malbranchea pulchella	+
Myriococcum albomyces	--
Pencillium duponti	+
P.purpurogenum	--
Talaromyces luteus	--
Thermoascus aurantiacus	--
Thermomyces lanuginosus GSLMBKU-10	+
Thermomyces lanuginosus GSLMBKU-11	+
Thermomyces lanuginosus GSLMBKU-12	+
Thermomyces lanuginosus GSLMBKU-13	+
Thermomyces lanuginosus GSLMBKU-14	+
Torula thermophila	--

(+) appearance of zone(positive)

(--) No clearance zone (negative)

Table 4. ANOVA of α- amylase production on different synthetic media by three strains of *T.lanuginosus*

Sources of variation	Sum of Squares	df	MS	F	P	Result
Between groups	27081.9259	2	13540.963	55.31	<.0001	S
Within Groups	45816.3148	17				
Total	81221.6481	53				

S- Significant

Table 5. ANOVA of Effect of pH on α- amylase production by three strains of *T.lanuginosus*

Sources of variation	Sum of Squares	df	MS	F	P	Result
Between groups	7893.5556	2	3946.7778	2.28	0.115424	S
Within Groups	173076.3175	20				
Total	250269.6508	62				

S- Significant

Table 6. ANOVA of Effect of Temperature on α- amylase production by three strains of *T.lanuginosus*

Sources of variation	Sum of Squares	df	MS	F	P	Result
Between groups	11264.9333	2	5632.4667	8.61	0.001218	S
Within Groups	77738.8	14				
Total	107318.8	44				

S- Significant

Table 7. ANOVA of Influence of different carbon sources on α- amylase production by three strains of *T.lanuginosus*

Sources of variation	Sum of Squares	df	MS	F	P	Result
Between groups	1222.9333	2	611.4667	1.05	0.00034	S
Within Groups	313680.4	44				
Total	366158.4	134				

S- Significant

Table 8. ANOVA of Influence of different Nitrogen sources on α- amylase production by three strains of *T.lanuginosus*

Sources of variation	Sum of Squares	df	MS	F	P	Result
Between groups	7462.1806	2	3731.0903	8.52	0.000398	S
Within Groups	255651.5556	47				
Total	304284.2222	143				

S- Significant

Table 9. ANOVA of β- amylase production on different synthetic media by three strains of *T.lanuginosus*

Sources of variation	Sum of Squares	df	MS	F	P	Result
Between groups	60742.3704	2	30371.1852	71.27	<.0001	S
Within Groups	26498.8148	17				
Total	101730.1481	53				

S- Significant

Table 10. ANOVA of Effect of pH on β- amylase production by three strains of *T.lanuginosus*

Sources of variation	Sum of Squares	df	MS	F	P	Result
Between groups	60653.1746	2	30326.5873	46.53	<.0001	S
Within Groups	203224.6032	20				
Total	289947.9365	62				

S- Significant

Table 11. ANOVA of Effect of Temperature on β- amylase production by three strains of *T.lanuginosus*

Sources of variation	Sum of Squares	df	MS	F	P	Result
Between groups	4448.5778	2	2224.2889	10.76	0.000341	S
Within Groups	153156.9778	14				
Total	163394.3111	44				

S- Significant

Table 12. ANOVA of Influence of different carbon sources on β- amylase production by three strains of *T.lanuginosus*

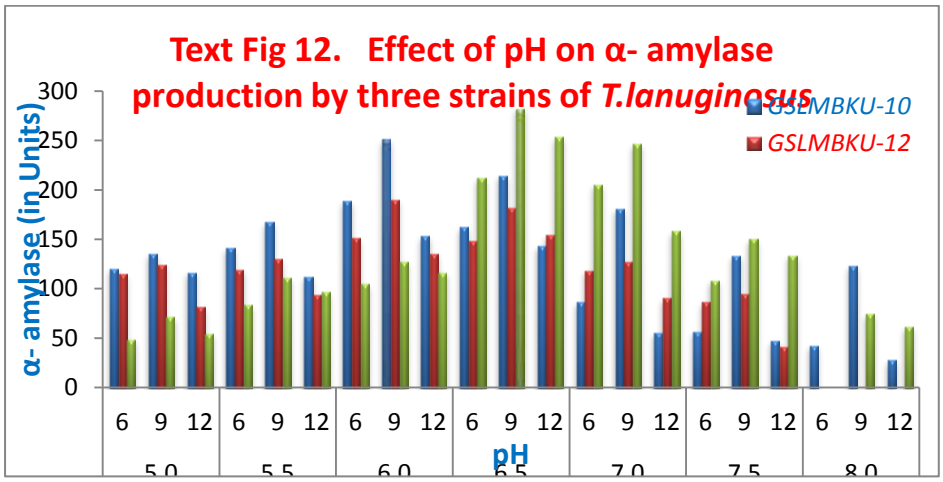
Sources of variation	Sum of Squares	df	MS	F	P	Result
Between groups	1540.5037	2	770.2519	2.98	0.00055943	S
Within Groups	331602.7704	44				
Total	355894.1037	134				

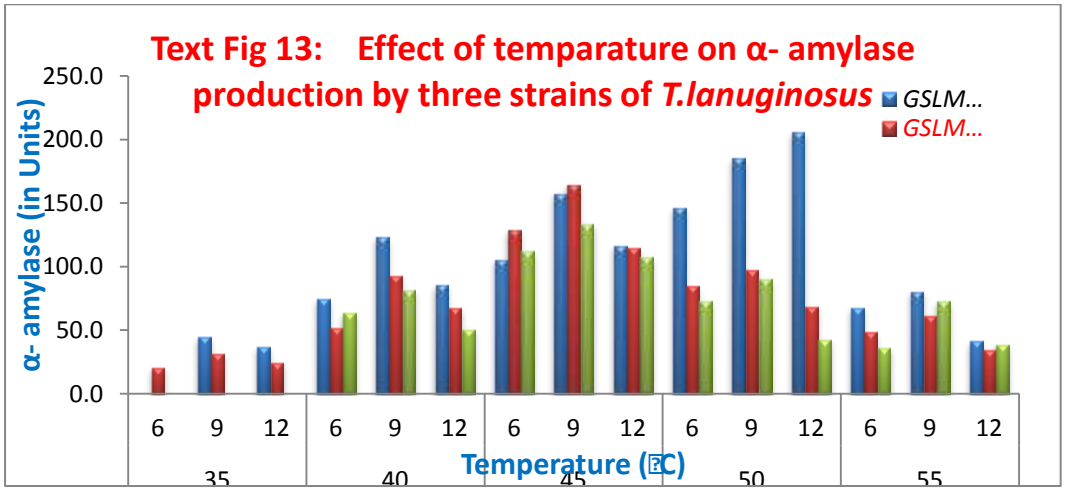
S- Significant

Table 13. ANOVA of Influence of different Nitrogen sources on β- amylase production by three strains of *T.lanuginosus*

Sources of variation	Sum of Squares	df	MS	F	P	Result
Between groups	15128.2222	2	7564.1111	20.58	<.0001	S
Within Groups	393599.5556	47				
Total	443270.8889	143				

S- Significant





CONCLUSION

GSLMBKU-10 could achieve good mycelial growth during its growth on L-asparagine and yeast extract, while it was least in medium containing L-glutamic acid. Glycine was more suitable nitrogen source for the vegetative growth of GSLMBKU-12, whereas ammonium sulphate was least preferred. Yeast extract and tryptophan were preferred nitrogen sources for maximum vegetative growth of GSLMBKU-14 and L-aspartic acid and urea was least preferred. Rest of nitrogen sources supported intermediate amount of mycelial growth. Twelve days incubation period was optimum for the vegetative growth of all the three strains under investigation.

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