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Effect of Various Factors on Growth and L- Asparaginase Production by Thermophilic Fungal Strains

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ABSTRACT:- Yeast extract followed by ammonium nitrate and L-arginine supported maximum production of asparaginase by GSLMBKU-10 while L-tyrosine, ammonium salts, L-methionine and L-histidine were poor nitrogen sources for the production of asparaginase. When asparaginase production by GSLMBKU-10 showed increasing trend with the progress of incubation period on L-glutamic acid, other nitrogen sources were responsible for decrease in asparaginase production after nine days of incubation period. GSLMBKU-12 secreted comparatively more amount of asparaginase during its growth in medium containing yeast extract, ammonium nitrate, glycine and sodium nitrate while it was low in medium containing aspartic acid and ammonium chloride. Rest of the nitrogen sources supported intermediate amount of asparaginase production. Nine days incubation period was optimum for production of asparaginase. Screened by ANOVA using fungal strains.

KEYWORDS: Yeast, asparaginase, ANOVA.

INTRODUCTION

L-asparaginase is a tetrameric protein belonging to oncolytic enzymes and it catalyzes the hydrolytic deamination of L-asparagine to L-aspartic acid and ammonia. L-asparaginase is known chemotherapeutic agent against cancer, such as acute lymphoblast leukemia and lymph sarcomas. These are used mainly in the treatment of children. Several reviews are available concerning the use of L-asparaginase in cancer therapy[1-4]. This enzyme causes selective death of asparagine dependent tumor cells and also induces apoptosis in tumor cells. It is probably best known growth inhibitory enzyme in clinical oncology. Another potentially important application of asparaginase is in the food industry for the production of acrylamide-free food [5-8].

Elspar, Oncaspar, Erwinase and kidrolase are trade names of L-asparaginase. Although Clementi has reported its presence in guinea pig serum, the anti-tumor properties[9-12] of the enzyme were recognized only sometime later. Tsujii first reported deamidation of L-asparagine in extracts of *E.coli* in 1957. Broome in 1961 discovered that his regression of lymphosarcoma transplants in mice treated with guinea pig serum was due to the nutritional dependence of the malignant cells on exogenous L-asparagine. Commercial production of L-asparaginase appeared desirable only after Mashburn and Wriston showed that L-asparaginase from *E.coli* inhibits tumors in mice[13-14].

Asparaginases are widespeard among bacteria, fungi, yeast (Oliveira *et al.*, 2003; , actinomycetes and plants .Several microbial strains like *Aspergillus tamari, Aspergillus terreus*, *Escherichia coli*, *Pseudomonas stutzeri*, *Pseudomonas aeruginosa*, *Vibrio succinogenes* and *Staphylococcus sp*. have been isolated and studied for their asparaginase production. L-asparaginase from bacterial sources produces hypersensitivity with long-term use, leading to allergic reactions and anaphylaxis. Whereas Eukaryotic microorganisms like yeast and filamentous fungi produces asparaginase with fewer adverse effects.

MATERIALS AND METHOD

These enzymes work optimally at 40-60˚C and at pH 6.0-7.0. Since baking temperature often goes up to 120˚C, it is desirable to have enzymes that are stable and active over a wide range of temperature and pH. Therefore, L-asparaginase from various sources (bacterial, fungal, plant, and animals) has been investigated.

Though L-asparaginase production by mesophilic fungi was investigated by large number of workers including Gulati *et al.* (1997), Seriquis *et al.* (2004), Lee *et al.* (2005) and Ferrara *et al.* (2006), only limited information is available on thermophilic fungal asparaginase. Hence, in the present studies L-asparaginase production by different strains of *T.lanuginosus* was investigated. All the strains are evaluated by ANOVA.

RESULTS AND DISCUSSION

Asparaginase 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. the growth of *GSLMBKU-14*, while *GSLMBKU-12* opted medium E followed by B and *GSLMBKU-10* opted medium B followed by A. twelve 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 *GSLMBKU-12* could grow in the pH range of 5.0 to 8.0. It failed to grow at pH 8.5. pH 6.0 was most suitable for enzyme production as well as mycelial growth followed by pH 6.5. *GSLMBKU-14* was able to grow in the pH range of 5.0 to 8.5 and pH 6.5 was optimum for asparaginase production. Almost similar trend was observed in *T.lanuginosus12*. pH 6.0 to 7.0 was optimum for all the three strains under investigation. Shown in Tables **1-5, from fig.33-35**.

Table-1 ANOVA of asparaginase 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	845.5926 4021.4259 5621.4259	2 17 53	422.7963	19.05	<.0001	S

S- Significant

Table-2 ANOVA of Effect of pH on asparaginase production by three strains of *T.lanuginosus*

Sources of variation	Sum of Squares	df	MS	F	P	Result
Between groups Within Groups Total	1336.1111 9479.2778 11843.9444	2 23 71	668.0556	29.88	<.0001	S

S- Significant

Table-3 ANOVA of Effect of temperature on asparaginase production by three strains of *T.lanuginosus*

Sources of variation	Sum of Squares	df	MS	F	P	Result
Between groups Within Groups Total	1426.037 7040.537 9563.8704	2 17 153	713.0185	22.09	<.0001	S

S- Significant

Table-4 ANOVA of Influence of different carbon sources on asparaginase production by three strains of *T.lanuginosus*

Sources of variation	Sum of Squares	df	MS	F	P	Result
Between groups Within Groups Total	3629.9704 32162.8593 41420.8593	2 44 134	1814.9852	28.38	<.0001	S

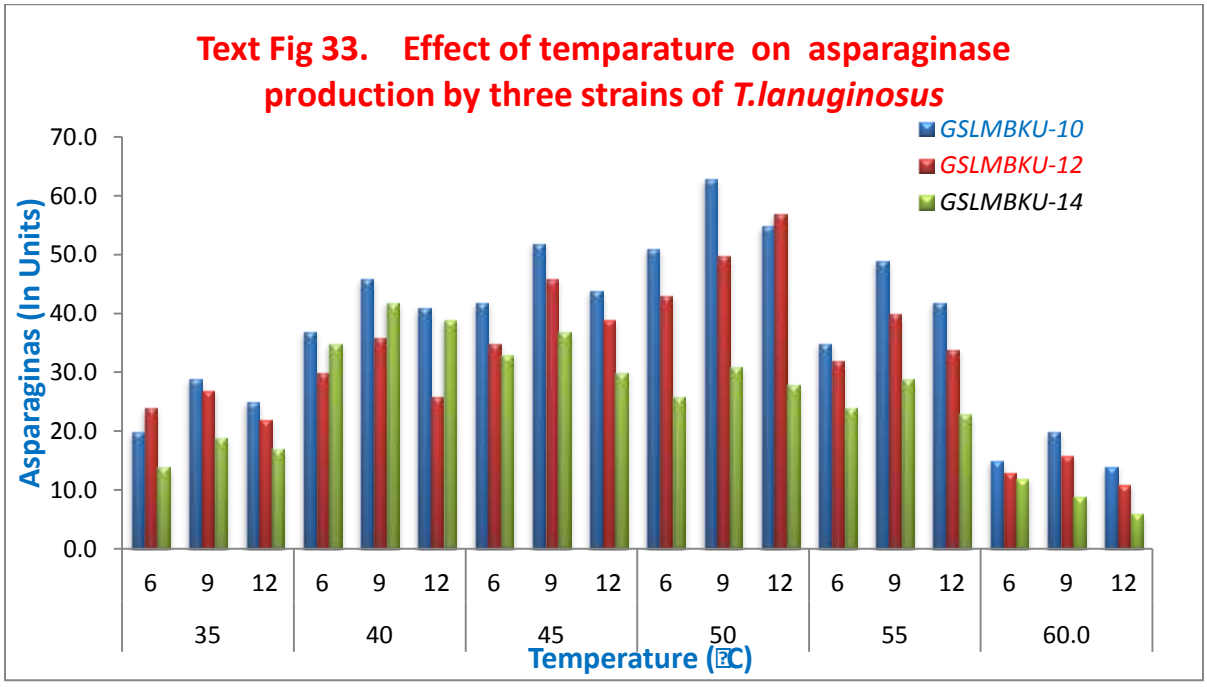
S- Significant

Table-5 ANOVA of Influence of different nitrogen sources on asparaginase production by three strains of *T.lanuginosus*

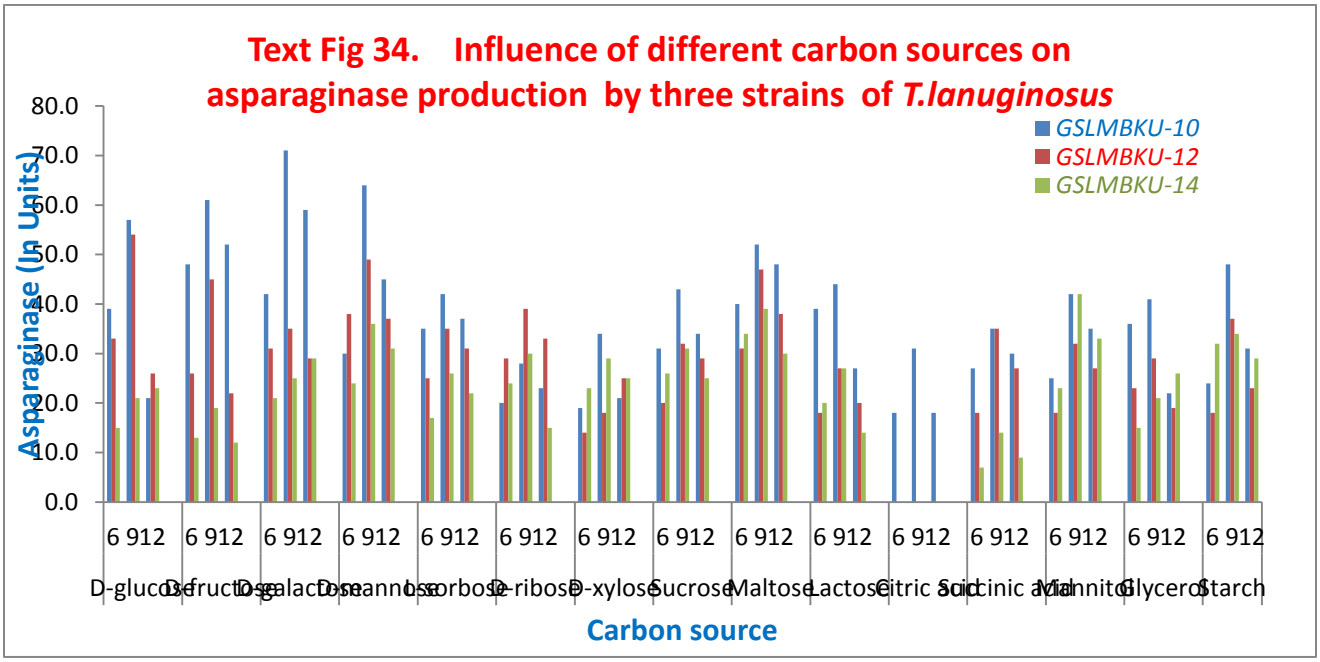
Sources of variation	Sum of Squares	df	MS	F	P	Result
Between groups Within Groups Total	247.3856 26291.3072 29916.6405	2 50 152	123.6928	3.66	0.00029	S

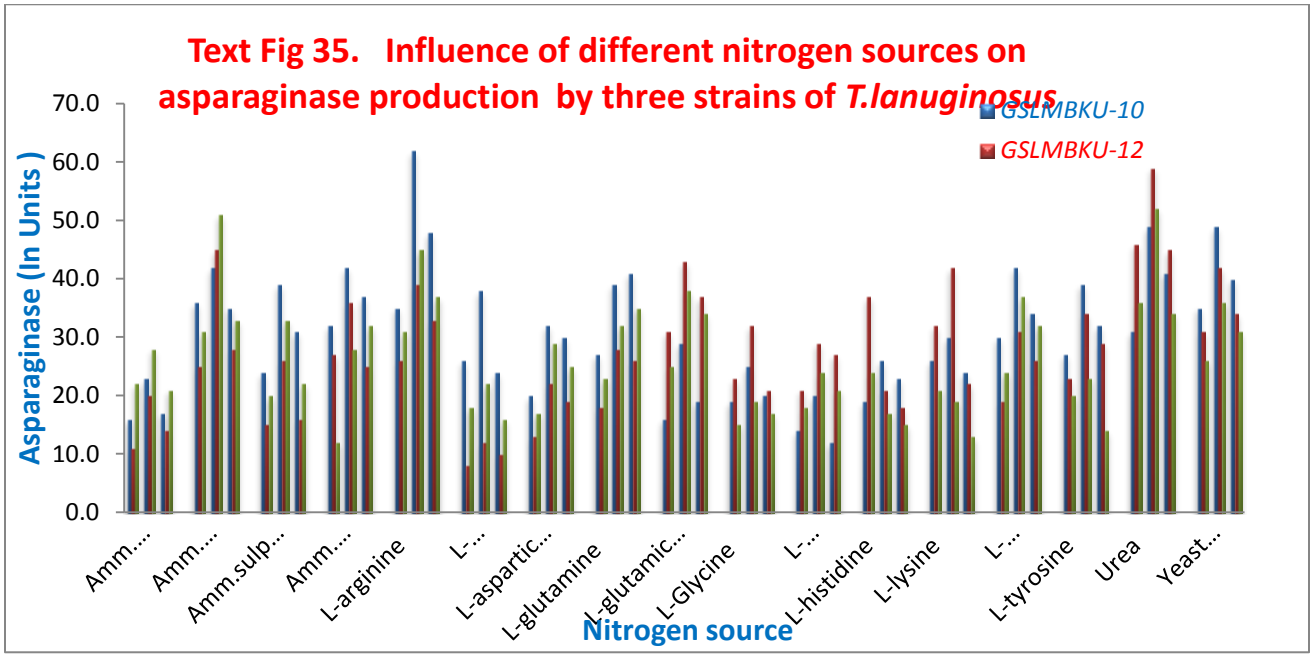
S- Significant

Text Fig 33. Effect of temparature on asparaginase production by three strains of *T.lanuginosus*



Text Fig 34. Influence of different carbon sources on asparaginase production by three strains of *T.lanuginosus*





CONCLUSION

GSLMBKU-10 could secrete good amount of asparaginase during its growth on L-arginine and L-asparagine, while it was low during its growth on histidine and ammonium chloride. *GSLMBKU-12* secreted comparatively more amount of asparaginase during its growth in medium containing yeast extract, ammonium nitrate, glycine and sodium nitrate while it was low in medium containing aspartic acid and ammonium chloride. Rest of the nitrogen sources supported intermediate amount of asparaginase production. Nine days incubation period was optimum for production of asparaginase. However, in medium containing methionine the enzyme production showed increasing trend till the end of incubation period tried.

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