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Role of Nitrogen Source for L-Glutaminase Production from Fungal Strain using through Submerged Fermentation

Krishna[1*]., Parimala B[2]., Harish Kumar T. S[3]. and Manjunath Basappa Channi[4]

*[*1]Department of Biotechnology, University College of Science, Tumkur University, Tumakuru, Karnataka, India*

[2]Department of Zoology, University College of Science, Tumkur University, Tumakuru, Karnataka, India

[3]Department of Zoology, D.R.M. Science College, Davangere, Karnataka, India

[4]Department of Zoology, D.R.M. Science College, Davangere, Karnataka, India

****Corresponding Author:** Dr. Krishna*

******Assistant Professor, Department of Biotechnology, University College of Science, Tumkur University, Tumakuru, Karnataka, India*

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Abstract

L-glutaminase has attracted much attention due its wide range of applications in several fields. The L-glutaminase widely used in pharmaceutical and food industries. L-glutaminase is generally regarded as a key enzyme that controls the delicious taste of fermented foods such as soy sauce. L-glutaminase production was carried out by using supplementation of organic and inorganic nitrogen sources such as yeast extract, malt extract, peptone and urea at concentration ranging from 0.25% to 1.25% with increments of 0.25% and also different inorganic nitrogen sources like ammonium sulphate and ammonium chloride at concentration ranging from 0.025% to 0.125% with increments of 0.025%. The malt extract (1%) produced 399.9 IU, were best organic nitrogen source and ammonium sulphate (0.1%) appear to be good inorganic nitrogen source under submerged fermentation process and showed 546 IU. Current study is an exploring step to industrial sector to upscale their L-glutaminase production and it will useful strategy to commercial sector and alternative to old methods.

Keywords: L-glutaminase, Organic, Inorganic, Submerged fermentation, Enzyme, Pharmaceutical

1. Introduction

Enzymes as drugs have two important features that distinguish them from all other types of drugs. First, enzymes often bind and act on their targets with great affinity and specificity. Second, enzymes are catalytic and convert multiple target molecules to the desired products. These two features make enzymes specific and potent drugs that can accomplish therapeutic biochemistry in the body that small molecules cannot. These characteristics have resulted in the development of many enzyme drugs for a wide range of disorders [1, 2]. Development of medical applications for enzymes have been at least as extensive as those for industrial applications, reflecting the magnitude of the potential rewards: for example, pancreatic enzymes have been

in use since the nineteenth century for the treatment of digestive disorders. The variety of enzymes and their potential therapeutic applications are considerable [3, 4].

L-glutaminase (L-glutamine amidohydrolase EC 3.5.1.2) is an industrially important hydrolytic enzyme catalyzes the hydrolysis of L-glutamine to L-glutamic acid and ammonia (Figure 1) [5]. Glutamic acid is known to be an important amino acid contributing not only to the pleasant taste "Umami", but also to the nutritional properties of food. Therefore, the addition of safe starter cultures containing glutaminase activity to fermented sausages is desirable because this enzyme is able to act on L-glutamine, present in relatively high amounts in the fresh mix, generating ammonia, as a neutralizer of acidity, and L-glutamate, as a flavor enhancer [6, 7].

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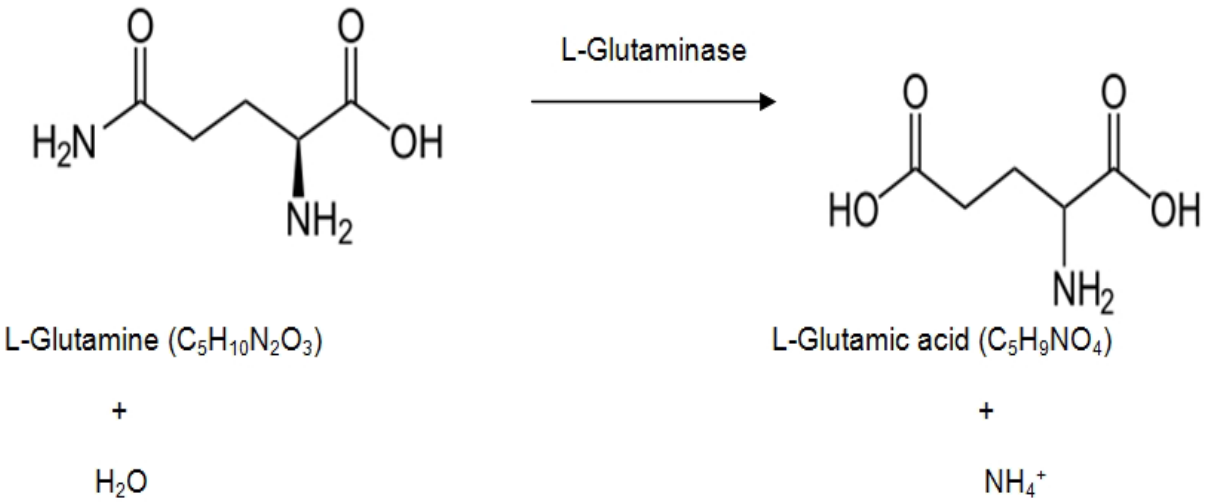

Figure 1: Schematic representation of mechanism of action of L- glutaminase

Glutaminase activity is widely distributed in plants, animal tissues and microorganisms including bacteria, fungi and yeast [8-10].

This article highlights on supplementation of nitrogen sources (both organic and inorganic nitrogen sources) for the synthesis of L-glutaminase from Aspergillus oryzae through submerged fermentation.

2. Materials and Methods

2.1 Fungal strain

Soil samples from different regions from Vijayawada, Andhra Pradesh such as Garden, Backyard, Agricultural and Near Coconut Tree. The collected soil samples were used for isolation of fungi by serial dilution method on Czepek Dox agar plate. The fungal strains tentatively identified in the laboratory as described by Shahid et al., [11] and were named as *Aspergillus oryzae* and maintained on Czapek Dox's agar (CZA) slants [12],

2.2 Screening of *Aspergillus oryzae* for L-glutaminase through Plate Assay

The plate assay carried out by modified the method of Gulati *et al*,[13]. The organisms were grown and kept on slants of solid modified Czapek Dox's medium. The plates were inoculated with 96-hr cultures of *Aspergillus oryzae* for screening of L-glutaminase observed for pink zone around the colony.

2.3 Production of L-glutaminase

Submerged fermentation was carried out for the production of L-glutaminase by employing *Aspergillus oryzae* KGSD 02 strain. The production medium consists of dextrose 0.1%, yeast extract 0.3%, KCl 0.02%, NaCl, 0.01%, $MgCl_2$ 0.02% and starch 0.5% w/v.

2.4 Supplementation of Nitrogen sources for L-glutaminase synthesis

2.4.1 Effect of Nitrogen source

Here both organic and inorganic nitrogen sources were supplemented for the better yield.

2.4.2 Effect of organic nitrogen source

A set of conical flasks with 100 ml of production medium supplemented with different organic nitrogen sources such as yeast extract, malt extract, peptone and urea at concentrations ranging from 0.25% to 1.25% with increments of 0.25%.

2.4.3 Effect of inorganic nitrogen source

A set of conical flasks with 100 ml of production medium with different supplemented with different inorganic nitrogen sources like ammonium sulphate and ammonium chloride at concentrations ranging from 0.025% to 0.125% with increments of 0.025%.

2.4.4 Assay of L-glutaminase for crude extract

Assay of L-glutaminase was carried out as per Imada et al., [14]. 0.5ml of 0.04M glutamine was taken in a test tube, to which 0.5 ml of 0.5 M buffer (acetate buffer pH 5.4), 0.5 ml of enzyme and 0.5 ml of distilled water was added to make up the volume up to 2.0 ml and incubate the reaction mixture for 30 min. After the incubation period the reaction was stopped by adding 0.5 ml of 1.5 M TCA (Trichloroacetic acid). 0.1ml was taken from the above reaction mixture and added to 3.7 ml distilled water and to that 0.2 ml Nessler' s reagent was added and incubated for 15 to 20 min. The optical density was measured at 450 nm. The blank was run by adding enzyme preparation after the addition of TCA. The enzyme activity was expressed in international unit.

2.5 International Unit (IU)

One IU of L–glutaminase is the amount of enzyme which liberates 1µmol of ammonia per minute per ml [µmole/ml/min].

3. Results and Discussion

In the present study, thirty strains of *Aspergillus oryzae* were isolated and named serially from KGSD 01 to KGSD30. All the thirty were screened for L-glutaminase production by plate assay. All strains of *Aspergillus oryzae* has been screened on the basis of the diameter of the pink zone. *Aspergillus oryzae* KGSD02 exhibited higher zone of diameter (1.2cm) and was considered as a potential strain for L-glutaminase production among the strains isolated from soil.

The organic nitrogen sources greatly influence the growth of any organism. In the present study various organic nitrogen sources like, yeast extract, malt extract and peptone were supplemented at 0.25%, 0.50%, 0.75%, 1% and 1.25% levels and urea were supplemented in the range of 0.025-0.1% to the synthetic medium for the production of L-glutaminase by *Aspergillus oryzae* KGSD 02. The results [Figure 1-4] revealed that the production of L-glutaminase increased with the increase in the organic nitrogen concentration up to 1.0%, thereafter no significant increase in L-glutaminase was noticed on all the days of fermentation with all nitrogen sources. Thus, nitrogen sources like yeast extract (1%) 310.5 IU, malt extract (1%) 399.9 IU and urea (0.05%) 181.2 IU peptone (1%) 208.2 IU influenced the L-glutaminase production respectively by *Aspergillus oryzae* KGSD 02 strain. Amongst various organic nitrogen sources tested, malt extract produced maximum 399.9 IU amount of L-glutaminase and emerged as best organic nitrogen source for the enhancement of L-glutaminase production by employing strain *Aspergillus oryzae* KGSD 02 under submerged fermentation.

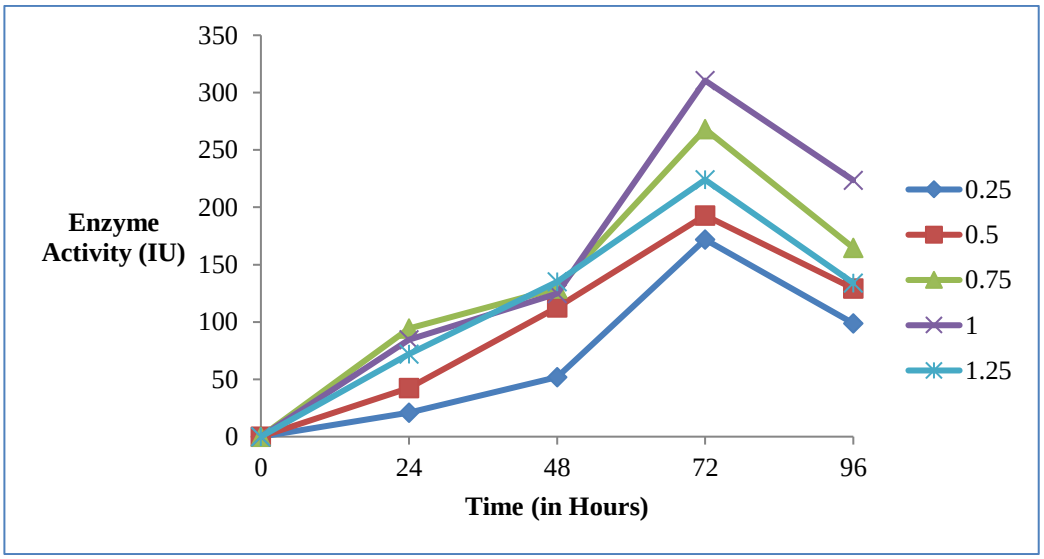

Figure 2: Effect of Yeast extract on L-glutaminase production

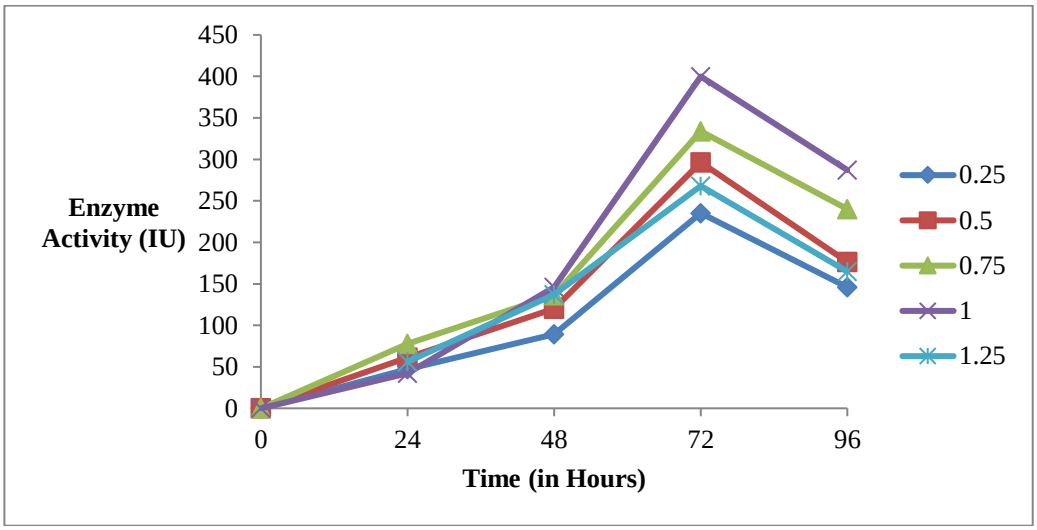

Figure 3: Effect of Malt on L-glutaminase production

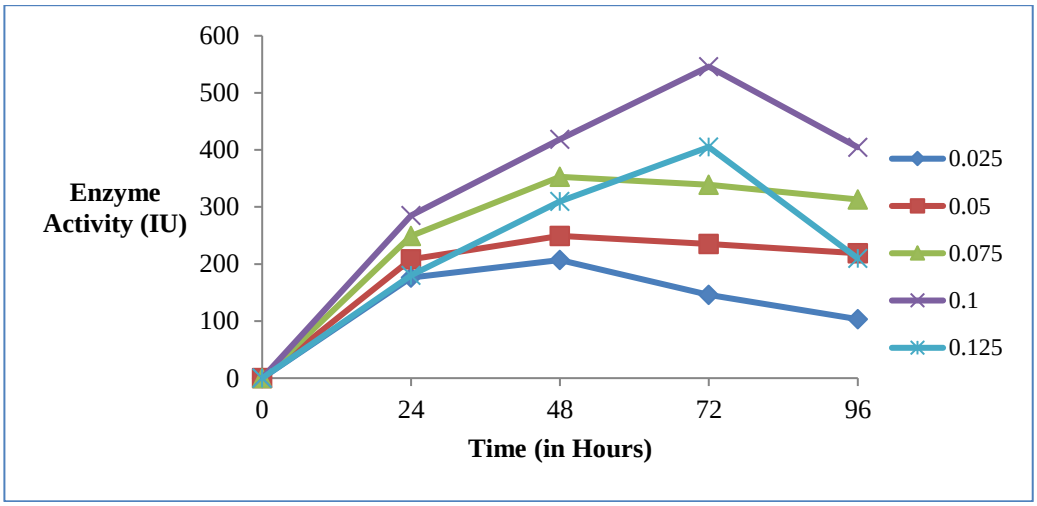

Figure4: Effect of NH_4Cl on L-glutaminase production

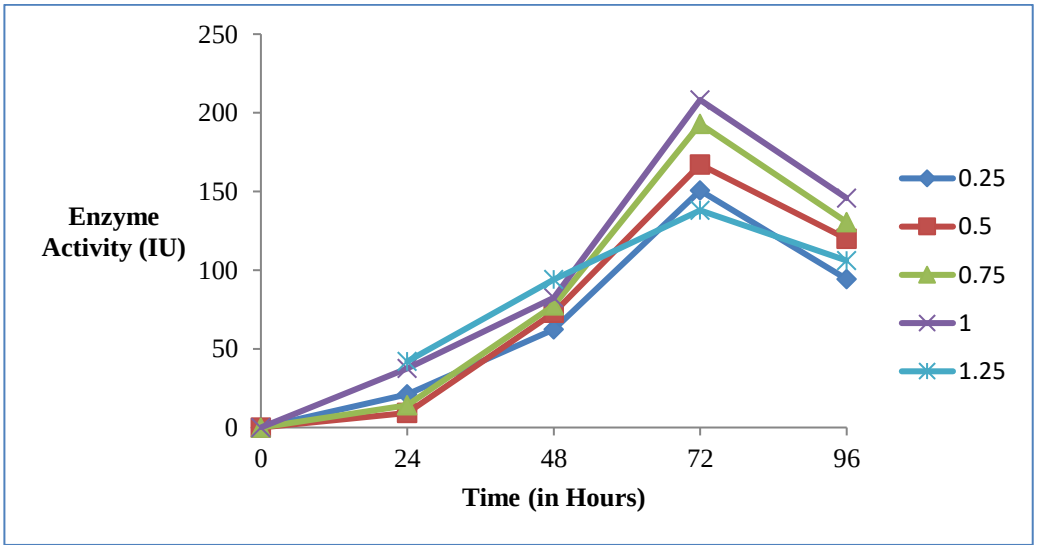

Figure 5: Effect of Peptone on L-glutaminase production

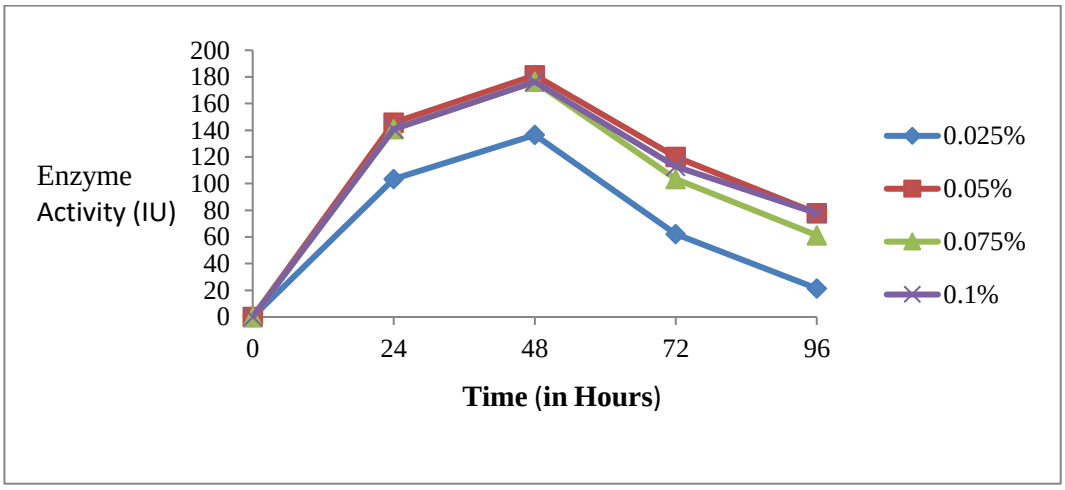

Figure 6: Effect of Urea on L-glutaminase production

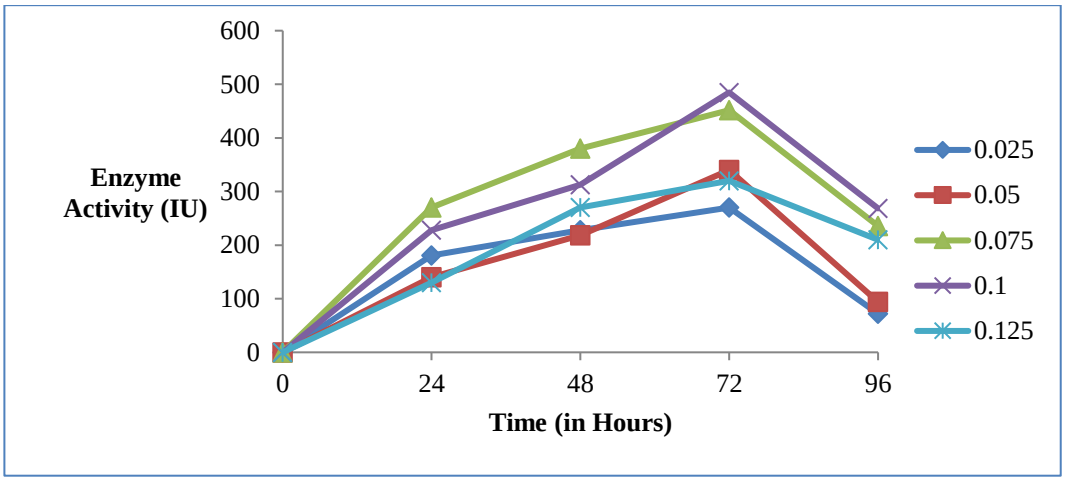

Figure 7: Effect of (NH_4) SO_4 on L-glutaminase production

The inorganic nitrogen source does impart growth and physiological activities of microorganisms. In the present study various inorganic nitrogen sources such as ammonium sulphate and ammonium chloride were used at various concentrations (0.025%, 0.05%, 0.075%, 0.1.%, 0.125%) and supplemented to the synthetic medium for the production of L-glutaminase by *Aspergillus oryzae* KGSD 02.

The results pertaining to the inorganic nitrogen studies are presented in Figure 5 and Figure 6 indicated that L-glutaminase production increased with the increase in the inorganic nitrogen concentration upto 0.1% and further increase in inorganic nitrogen source has revealed no significant change in L-glutaminase production. The highest production of enzyme 546 IU and 484.8 IU was observed by supplementation of ammonium chloride and ammonium sulphate respectively by employing *Aspergillus oryzae* KGSD 02 strain on production medium at 72 hrs of fermentation period. Thus, amongst all inorganic nitrogen sources provided for L-glutaminase production, ammonium sulphate (0.1%) appears to be good inorganic nitrogen source under submerged fermentation process.

The requirement of nitrogen source mainly depends on the strain and the substrate employed during fermentation [15]. Nitrogen can be a limiting factor in the microbial production of enzymes [16]. Chanakya *et al*., [17] were showed that, among the different nitrogen sources (peptone, yeast extract, malt extract and beef extract) tested, Malt extract (1% w/v) was the best source for maximal enzyme production (19.41 U/g of dry substrate) by using *Trichoderma koningii*. Optimal malt extract concentration was studied by varying its concentration in the medium and maximum enzyme production was observed at the same concentration of 1.0% (w/v).

Reports of Abdallah *et al.*, [18] were described the effect of inorganic nitrogen sources are in the range 0.08% of sodium nitrate, ammonium nitrate and ammonium sulphate were used for the production of L-

glutaminase by employing *Streptomyces avermitilis*. Among these sources ammonium nitrate was showed best in-organic nitrogen and it produce 10.30 U/ml and ammonium sulphate showed 3.04 U/ml.
In the study of Jayabalan *et al*., [19] state that, among the inorganic nitrogen and salt sources tested, only potassium dihydrogen phosphate was found to enhance the L-glutaminase production (44.3 U/ml). Ammonium sulphate, sodium nitrate and calcium nitrate were found to decrease the enzyme production at 1%, w/v concentration, but sodium nitrate evolved better in-organic nitrogen source by using *B. diminuta* and it showed 41.2 ±0.5U/ml. Such studies also reported as extensive review by various authors in the book Enzyme – Mechanism and Action to illustrate their various applications and sources to production [20].

4. Conclusion

Many strains were isolated from different locations yielding a total of 30 strains, among them 02 were positive for L-glutaminase production when subjected to rapid plate assay. The results of the best five strains are reported for the industrial application in various fields. As per the results strain identified as *Aspergillus oryzae* emerging as a best strain among the studied organisms. Further studies on the optimization of physical, chemical, nutritional parameters and molecular characterization are being carried out to know more about these potential organisms and their wide range of application in various fields to make best commercial use as an alternative to previous strategies.

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Conflict of Interest

The authors declare no conflict of interest.

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