



## Optimization of inducer concentration in the medium for production of Glutaminase from *Erwinia*

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### Abstract:

The purpose of study was to determine effect of inducer concentrations in the medium for production of Glutaminase enzyme from *Erwinia*. For production of glutaminase enzyme from free cells of *Erwinia*, various physical and chemical parameters were optimized. The microorganism *Erwinia* which is a source of glutaminase was procured from Institute of Microbial Technology, Chandigarh and was grown on specified medium. The media was sterilized by autoclaving for 15 minutes at 15 psi/cm<sup>2</sup>. The microorganism was maintained on agar slants and kept in refrigerator at 4.0 + 0.5 °C for further use and was sub-cultured after every 20 days. The viability check of microorganism was done by gram staining and tryphan blue. The work utilized Beef extract, Peptone, Yeast extract, Sodium chloride and Water as composition of growth medium for the production of glutaminase enzyme. The parameters such as Temperature, pH, & Time were optimized. By using these parameters Glutaminase was produced by adding different concentration of inducers such as Ammonium sulphate, Inorganic phosphate & L-Glutamine. It was concluded that as we increase the concentration of inducers, enzyme activity increases up to a certain concentration. The enzyme activity was determined by Nessler's reagent method. So it is clear from this study that Inducers are very helpful to increase the production of glutaminase enzyme from *Erwinia* and after adding inducers Ammoniumsulphate 100mM, Inorganic phosphate 200iL & L- Glutamine 12.5% the enzyme production was maximum.

**Key Words:** *Glutaminase Enzyme, Erwinia, Inducers*

### Introduction:

The protein glutaminase is traditionally considered as a mitochondrial enzyme, playing a key role in the energy and nitrogen metabolism of mammalian cells. The microbial enzymes are found to play a major role in the diagnosis, cure, biochemical investigation, and in the monitoring of many dreadful diseases. Microorganisms represent an excellent source of many therapeutic enzymes owing to their broad biochemical diversity and their susceptibility to

genetic manipulation. Glutamine, out of the 20 amino acids is commonly found in animal proteins which supplies energy and substrate to the host after severe stress<sup>[1]</sup> Only the L-stereoisomer is found to occur in mammalian protein. The liver shows net glutamine uptake after a protein-containing meal during uncontrolled diabetes, sepsis and short-term starvation but changes to net release during long-term starvation and metabolic acidosis<sup>[2]</sup>. Glutamine structure is identical to that of glutamic acid except that the acidic side-chain carboxyl group of glutamine has been coupled with ammonia, yielding an amide. The glutamic acid-glutamine inter conversion is of central importance to the regulation of the level of toxic ammonia in the body. Thus, it is not surprising that when the concentration of the amino acids of blood plasma is measured, glutamine is found to have the highest value. The role of glutamine in the prevention of chemotherapy and radiation-induced toxicity is evolving. The glutamine supplementation is inexpensive and is found to reduce the incidence of gastrointestinal, neurologic, and possibly cardiac complications during cancer therapy<sup>[3]</sup>. By enhancing the level of L-glutamine, the maximum glutaminase activity was obtained. Several mammalian and microbial glutaminase enzymes have been purified and characterized which appeared to be best for therapeutic use because of its low  $K_M$  for glutamine (micro molar range), good stability its activity and long plasma half-life in tumor-bearing hosts. The administration of enteral or parenteral glutamine seems to be safe and also potentially efficacious in some patient groups undergoing intensive treatment for cancer<sup>[4]</sup>. Glutaminase can be isolated from different microbial, plant and animal sources. The microbial sources from which glutaminase has been extracted are *Bacillus licheniformis* A5, *Micrococcus luteus* K-3, *Erwinia* species. *Erwinia* is a genus of Enterobacteriaceae containing mostly plant pathogenic species which was named by the first phyto bacteriologist, Erwin Smith. It is a gram negative bacterium related to *E.coli*, *Shigella*, *Salmonella* and *Yersinia*<sup>[5]</sup>

**Materials & Methods:**

Optimization of inducer concentration in the medium for the production of glutaminase.

Inducers used were:

1. Ammonium sulphate
2. Inorganic phosphate
3. L- Glutamine

Inducers are found to increase the enzyme production so their concentrations were optimized by adding different concentrations in the production medium, The effect on the enzyme activity in the supernatant was determined by using different concentrations of Ammonium sulphate, Inorganic phosphate and Glutamine. The concentrations used were as follows:

- **Ammonium sulphate:**  
100,150,200,250,300,350,400mM
- **Inorganic phosphate:**  
50, 75,100,150,200,250,300µl
- **3. Glutamine:**  
0.1, 0.2, 0.3, 0.4, 0.5 0.6g

**Results & Discussion:**

**1.1 Effect of ammonium Sulphate in the medium on glutaminase production**

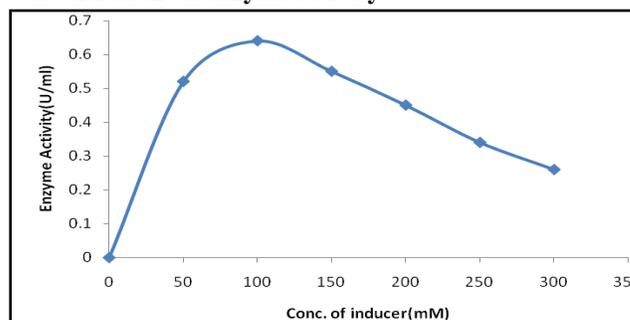
To find out the optimum concentration of ammonium sulphate in the production medium, different concentrations of ammonium sulphate (up to 400mM) were added into the medium.

**Table: 1.1 Effect of ammonium sulphate in the medium on glutaminase production**

S No.	Conc. of Ammonium sulphate(mM)	O.D at 630 nm	Enzyme Activity(U/ml)
1.	50	0.52	1832.71
<b>2.</b>	<b>100</b>	<b>0.64</b>	<b>2207.75</b>
3..	150	0.55	1916.22
4.	200	0.45	1582.2
5.	250	0.34	1207.16
6.	300	0.26	958.11

There was found a continuous increase in enzyme production with increase in the ammonium sulphate concentration in the medium. The maximum enzyme production was observed with 100mM concentration of ammonium sulphate.

**Fig.1.1 Standard curve showing effect of inducer concentration on enzyme activity**



It was observed that inducer (ammonium sulphate) concentration of 100mM showed maximum enzyme production. The inducer was found to increase the enzyme activity as inferred from the work done by other researchers but *B. licheniformis* did not show any significant effect on production of enzyme [6]

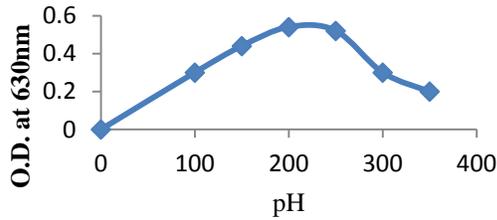
**1.2 Effect of inorganic phosphate in the medium on the production of glutaminase:**

S No.	Conc. of inorganic phosphate(µL)	O.D at 630 nm	Enzyme Activity(U/ml)
1.	100	0.38	1333.15
2.	150	0.44	1541.18
<b>3.</b>	<b>200</b>	<b>0.54</b>	<b>1873.73</b>
4.	250	0.52	1832.71
5.	300	0.50	1749.21
6.	350	0.20	748..61

**Table 1.2 2 Effect of inorganic phosphate in the medium on glutaminase production:**

To determine the optimum concentration of inorganic phosphate in the production medium, different concentrations of inorganic phosphate (ul) were taken. The continuous increase in enzyme production was observed with increase in the concentration of inorganic phosphate in the medium. The maximum enzyme production was observed with 200µL concentration of inorganic phosphate. Dura, 2001 observed no significant effect on glutaminase activity after addition of inorganic phosphate.

**Fig 1.2 Standard curve showing the effect of iP on Glutaminase production**



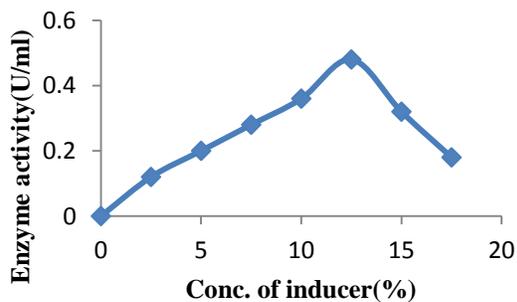
**1.3) Effect of glutamine in the medium on the production of Glutaminase**

There was a continuous increase in enzyme production with the increase in the concentration of glutamine in the medium. The optimum concentration was observed when 12.5% of glutamine was added in the production medium. In various microorganisms L-glutamine was observed to be good inducer for enzyme production because l-glutamine acted as sole source of nitrogen and carbon

**Table: 1.3 Effect of glutamine in the medium on Glutaminase production**

S No.	Conc.of Glutamine(%)	O.D at 630 nm	Enzyme Activity(U/ml)
1.	2.5	0.12	457.08
2.	5.0	0.18	666.57
3.	7.5	0.24	874.60
4.	10.0	0.34	1207.16
5.	12.5	0.48	1665.70
6	15.0	0.32	1123.65
7	17.5	0.18	666.57

**1.3 Standard curve showing inducer effect on Glutamine conc.**



**Conclusion:**

It was concluded from the above study that there was a continuous increase in enzyme production with increase of inducer concentration in the medium up to a certain level. After that there is slight decrease in enzyme production. The optimum concentration of inducers at which enzyme production was maximum was found as Glutamine 12.5%, ammonium sulphate 100mM & 200µL inorganic phosphate. As Glutaminase has many industrial and medicinal applications, this study must be helpful for further research

**Acknowledgement:**

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**Reference:**

[1] Houdijk, Alexander P J; Meijer, Sybren; Nijveldt, Robert J; van Leeuwen, Paul A M; Boelens, Petra G.(sep. 2001). Glutamine alimention in catabolic state. The Journal of Nutrition (High beam Encyclopedia).

[2] Watford and Malcolm (2000). Glutamine and glutamate metabolism across the liver sinusoid. The Journal of Nutrition (High beam Encyclopedia).

[3] Diane,M.F., Savy,G., Vahdat,L., Wischmeyer, P.E. and Corey.B (2003).Prevention of chemotherapy and radiation toxicity with glutamine. Cancer treatment reviews. vol.29:pp501-513.

[4] Ziegler, Tr., (2001). Glutamine supplementation in cancer patients receiving bone marrow transplantation and high dose chemotherapy. *J. Nutr.* Vol. 131: pp 2578s-84s.

[5] en.wikipedia.org/wiki/Glutamine).

[6] Cook,W.R., Hoffman, J.H. and Bernlohr,R.W. (1981).Occurrence of an Inducible glutaminase in *Bacillus licheniformis* .*Journal of bacteriology.* vol.148(1): pp 365-367.

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