Journal of Applied Pharmaceutical Science Vol. 3 (06), pp. 132-135, June, 2013
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2013.3622
ISSN 2231-3354

An approach on microbial biosynthesis of L-glutaminase: a tumour inhibitor

Sunil Dutt P.L.N.S.N.[1], Siddalingeshwara K.G[2], Karthic J[1], Mohsin S. Mushtaq[1], Naveen M[1] Vishwanatha T[3] and Prathiba K.S[4]

[1]Research and Development Centre Bharathiar University, Coimbatore, India.
[2]Padmshree Institute of Information Science Nagarabhavi Circle, Bangalore, India.
[3]Dept of Microbiology, Maharani Science College for Women, Bangalore, India.
[4]Dept. of Biotechnology, K.L.E Society's S. Nijalingappa College, Bangalore, India.

ARTICLE INFO

Article history:
Received on: 10/05/2013
Revised on: 24/05/2013
Accepted on: 05/06/2013
Available online: 27/06/2013

Key words:
L-glutaminase, fermentation kinetics, antitumour agent and *Aspergillus oryzae.*

ABSTRACT

L-Glutaminase, an amidohydrolase enzyme has been a choice of interest in the treatment of lymphoblastic leukemia. This study investigates the production of extracellular L-glutaminase synthesis were carried out by using Aspergillus oryzae was evaluated under different fermentation parameters by employing submerged fermentation method. The L-glutaminase producers detected by the pink zone around the colony by simple plate assay method. Aspergillus oryzae S2 is the potential strain among the fungal isolates. The L-glutaminase synthesis were increased their yield after the optimization of fermentation parameters. The optimum pH 5.0, temperature 350C and inoculum size 1.0 ml and it showed 217.65 IU.

INTRODUCTION

In recent years, biomedical sciences accentuate the involvement of glutaminase and other amino acid-depleting enzymes as agents for treating tumors (Holcenberg 1982). The tumor cells display tremendous dependence on the exogenous supply of L-glutamine as a growth substrate, since they do not demonstrate the L-glutamine synthetase (Rohde *et al.*, 1996).

The ongoing challenge of the tumor cells on its survival can be blocked by the action of L-glutaminase (EC 3.5.1.2), an amidohydrolase enzyme which generates L-glutamic acid and ammonia from L-glutamine (Sathish and Prakasham 2010) and It play substantial contributory role in cellular nitrogen metabolism in all living cells (Sathish and Prakasham 2010). The microbial production of metabolites and enzymes mainly depend on the genetic nature of the organism, fermentation medium components and their concentration, physiological growth conditions and interactive influence of all the above factors. Hence optimization of the above conditions is vital in order to get higher yields and to develop effective bioprocess system for industrial application. Many authors reported that the increased enzymes yield upon optimization of bioprocess conditions using different fermentation strategies (Sathish and Prakasham 2010, Sathish and Prakasham 2010, Hymavathi *et al.*, 2010 and Mahalaxmi *et al* 2009).

A variety of microorganisms, including bacteria, yeast, moulds and filamentous fungi have been reported to produce L-glutaminase (Kashyap *et al.*, 2002, Weingand-Ziade *et al.*, 2003 and Iyer and Singhal 2008) of which the most potent producers are fungi (Balagurunathan *et al.*, 2010). On an industrial scale, glutaminases are produced mainly by *Aspergillus* and *Trichoderma* sp (Tomita *et al.*, 1988, Masuoa *et al.*, 2004, El-Sayed 2009 and Palem *et al.*, 2010).

The objective of this study was to utilize *Aspergillus oryzae* with good ability to produce L-glutaminase by optimizing fermentation kinetics for production of maximizing the L-glutaminase synthesis.

* *Corresponding Author*
Dr.Siddalingeshwara K.G
Padmshree Institute of Information Science Nagarabhavi Circle, Bangaloe-10, India. Ph.+91 9449589140

MATERIALS AND METHODS

Chemicals

Glutamine used in the study was procured from Hi-Media Laboratories, Mumbai, India; the other ingredients used for the preparation of Czapek Dox's media were also products of Hi-Media Laboratories, Bombay.

Fungal strain

The *Aspergillus oryzae* strains were isolated from different soils. Soils are taken from different regions from Vijayawada. Tentatively identified in the laboratory and further the strains were identified at Agarkar research Institute (ARI), Pune.

Screening and Fermentation Medium

Aspergillus oryzae strains were screened for their L-glutaminase activity by plate assay and among the twenty five isolates, *Aspergillus oryzae* S2 were used for further studies. The selected *Aspergillus oryzae* S2 were cultured on production medium. 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.

Optimization Studies

The 250 ml Erlenmeyer flasks containing 100 ml of production medium were prepared by mixed with acid/alkali solution to obtain required pH. The pH was adjusted in the range of 3-7 with increments of 1.0.

Thus prepared flasks were cotton plugged and autoclaved at 121^{0}C for 15 min. The flasks were inoculated and incubated. The 100ml of the production medium was separately taken in 250 ml Erlenmeyer flasks and prepared for submerged fermentation. Thus prepared flasks were incubated at different temperatures like 25-40^{0}C with in increments of 5^{0}C.

The inoculum was prepared separately by reviving the 168h old culture of *Aspergillus oryzae* S2 at different levels i.e., 0.25, 0.50, 0.75, 1.0 and 1.25 ml and then fermentation studies were carried out.

Extraction of L-glutaminase

The samples were withdrawn periodically at 24 hrs in aseptic condition. The extract was filtered through Whatman filter No.1.

The clear extract was centrifuged at 2000-3000 rpm for 15 min, supernatant were used as enzyme preparation. Thus prepared crude enzyme was used for assay of L-glutaminase.

Assay of L-glutaminase

Assay of L-glutaminase was carried out as per Imad et al., (1973). L-glutamine were used as a substrate and the product ammonia were released during catalysis and it was measured by using Nesseler's reagent. The enzyme activity was expressed in International unit.

International unit (IU)

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

RESULTS AND DISCUSSION

Fungal isolates were identified as *Aspergillus oryzae* in Agrakar Research Institute, Pune. All twenty five strains of *Aspergillus oryzae* produced pink zones on glutamine plate medium; those were selected from the soil sample. Of the twenty five isolates *Aspergillus oryzae* S2 was considered to be the best and high L-glutaminase producing strain. It showed 2.23 cm of cleared zone around the colony. The data obtained in the present study on the effect of pH and temperature on submerged fermentation is shown in (Fig. 1 and 2) which reveals that the production of L-glutaminase increased with the increase in the pH of the medium up to pH 5.0 temperatures 35^{0}C and thereafter the decrease of L-glutaminase was observed.

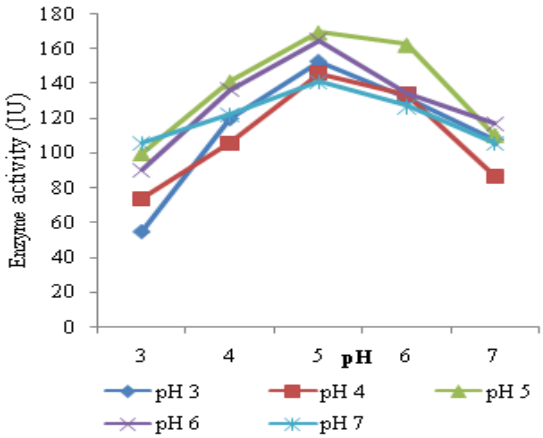

Fig. 1: Effect of pH on L-glutaminase production.

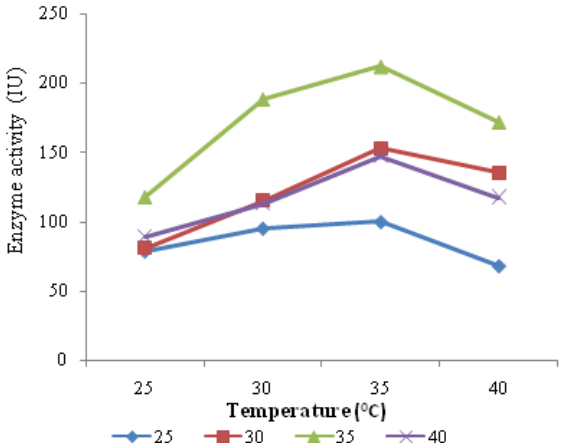

Fig. 2: Effect of temperature on L-glutaminase production.

The maximum production of L-glutaminase is 169.17 IU was obtained at pH 5.0 and the minimum production of L-glutaminase 141.17 IU was observed at pH 7.0. The production of L-glutaminase increased significantly with the increase in fermentation temperature from 25-35^{0}C and decreased above 35^{0}C.

The maximum L-glutaminase production obtained at 35^{0}C was 211.76 IU and the least production was observed at 25^{0}C resulted only 100 IU of L-glutaminase at 72 hrs of fermentation period. Any temperature beyond the optimum range is found to have some adverse effect on the metabolic activities of the microorganisms and it is also reported by various scientists that the metabolic activities of the microbes become slow at lower or higher temperature (Okolo *et al.*, 1995).

The pH of the medium is one of the most critical environmental parameter affecting the mycelial growth, enzyme production and the transport of various components across the cell membrane (Kapoor *et al.*, 2008).

In our study, the data revealed that the pH of 5.0 was found as suitable for maximum production of L-glutaminase with *Aspergillus oryzae* S2 strain under submerged fermentation. Fungal strains are noted for their best performance in the range of 3.5-7.0 and also low pH avoids the contamination by other microbes (Pandey *et al.*, 2001). Our findings are in close agreement with the earlier findings of Nathiya *et al* (2011), they showed that pH 6 was the suitable for maximum L-glutaminase production.

Incubation temperature dependent variation in L-glutaminase production was reported in several microbial species (Sivakumar *et al.*, 2006 and Keerthi 1999**)** Keeping this in view, experiments were conducted to understand the effect of temperature on L-glutaminase production by *Aspergillus oryzae* S2. The present study revealed that the 35 ^{0}C is suitable and maximum production of L-glutaminase with *Aspergillus oryzae* S2. Sateesh and Prakasham (2012) reported that the maximum production of L-glutaminase was observed at temperature 37 ^{0}C by using *Bacillus subtilis* RSP-GLU and it showed 167 U/ ml, similar observations were reported for glutaminase from *Trichoderma koningii* which produced 15.59 U/gds at 33°C (Pallem et al. 2010). Rajeev Kumar and Chandrashekaran reported 35 ^{0}C is the suitable for L-glutaminase production through submerged fermentation by using Pseudomonas sp BTMS-51 in packed bed reactor. As such our findings are close agreement with Rajeev Kumar and Chandrashekaran **(25).**

Importance of inoculum size on microbial fermentation process is widely accepted. Out of five inoculum size tested (0.25, 0.50, 0.75, 1.0 and 1.25 ml) and 1.0 ml inoculum was found to be the most suitable for high production of L-glutaminase by *Aspergillus oryzae* in submerged fermentation at 72 hrs of fermentation. From Fig. 3, it is clear that the L-glutaminase production steadily increased with the increasing in the size of the inoculum until it reaches to the magnitude when enzyme productivity became maximum, thereafter no appreciable change in production of L-glutaminase with high inoculum size could be observed. The maximum enzyme activity was showed at 217.65 IU. at 1.0 ml inoculum size and least enzyme activity 74.11 IU was showed at 0.25 ml of inoculum size. Nathiya *et al* (2011) reported that 2ml of fungal spores as an inoculum. The maximal glutaminase production (14.19 U/g of dry substrate) was observed when an inoculum concentration of 2ml of 6 day old fungal culture was added. At lower and higher inoculum levels, poor glutaminase production was observed. It is very important to provide an optimum inoculum level in fermentation processes were reported by Pallem *et al.*, (2010). Sateesh and Prakasham (2012) were showed inoculum concentration from 1.0 to 3.0 % and the L-glutaminase activity was monitored during growth phase of isolated *Bacillus subtilis* RSPGLU. The maximum enzyme production (176 U ml-1) was observed in 2.0% of initial inoculum supplemented conditions.

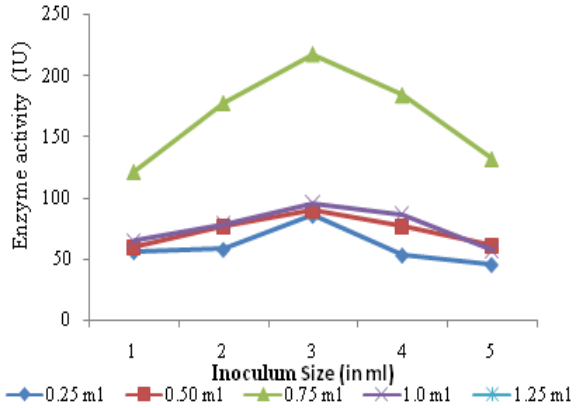

Fig. 3: Effect of inoculum Size on L-glutaminase production.

REFERENCE

Holcenberg JS. Enzyme Therapy: Problems and Solutions. Ann Rev Biochem 1982; 51: 795-812.

2. Rohde T., MacLean DA., Klarlund PD. Glutamine, lymphocyte proliferation and cytokine production. Sci J Immunol. 1996; 44: 48-650.

Kumar SR., Chandrasekaran M. Continuous production of L-glutaminase by an immobilized marine *Pseudomonas* sp. BTMS-51 in a packed bed reactor. Proc Biochem 2003; 38: 1431–1436.

Sathish T., Prakasham RS. Enrichment of glutaminase production by *Bacillus subtilis* RSP-GLU in submerged cultivation based on neural network - genetic algorithm approach. Journal of Chemical Technology and Biotechnology. 2010; 85(1):50-58.

Sathish T., Prakasham RS. Isolation and Identification of L-Glutaminase an antileukemic enzyme Producing Micro-organism from Godavari River Bank Soils in Andhra Pradesh. International Research Journal of Pharmacy. 2010; 1(1):367-373.

Hymavathi M., Sathish T., Brahmaiah P., Prakasham RS. Impact of Carbon and Nitrogen Sources on L-Asparaginase Production by Isolated *Bacillus circulans* (MTCC 8574): Application of Saturated Plackett-Burman Design. Chemical and Biochemical Engineering Quarterly. 2010; 24(4):473-480.

Mahalaxmi Y., Sathish T., Prakasham RS. Development of bala nced medium composition for improved rifamycin B production by isolated *Amycolatopsis* sp. RSP-3. Letters In Applied Microbiology. 2009; 49(5):533-538.

Kashyap P., Sabu A., Pandey A., Szakacs G., Soccol CR. Extracellular L-glutaminase production by *Zygosaccharomyces rouxii* under solid-state fermentation. Proc. Biochem. 2002; 38: 307-312.

Weingand-Ziade A., Gerber-Decombaz C., Affolter M . Functional characterization of a salt and thermo tolerant glutaminase from *Lactobacillus rhamnosus.* Enzyme Microb. Technol. 2003; 3: 862-867.

Iyer P., Singhal RS . Production of glutaminase (E.C.3.2.1.5) fr om *Zygosaccharomyces rouxii:* Statistical optimization using response surface methodology. Bioresour. Technol. 2008; 99: 4300–4307.

Balagurunathan R., Radhakrishnan M., Somasundaram S. L-glutaminase producing Actinomycetes from marine sediments –

selectiveisolation, semi quantitative assay and characterization of potential strain. Aust. J. Basic Appl. Sci. 2010; 4: 698-705.

Tomita K., Yano T., Kumagai H., Tochikura T. Formation of γ-Glutamylglycine by extracellular glutaminase of *Aspergillus oryzae*. J. Ferment. Technol. 1988; 66 (3): 299– 304.

Masuoa N., Itob K., Yoshimunea K., Hoshinoa M.., Matsushimab H, Koyamab Y, Moriguchia M. Molecular cloning, over expression, and purification of *Micrococcus luteus* K-3-type glutaminase from *Aspergillus oryzae* RIB40. Protein Exp. Purif. 2004; 38: 272–278.

El-Sayed ASA. L-glutaminase production by *Trichoderma koningii u*nder solid state fermentation. Ind. J. Microbiol. 2009; 4: 243–250.

Pallem C., Manipati S., Somalanka SR. Process optimization of L-glutaminase production by *Trichoderma koningii* under solid state fermentation (SSF). Int. J. Appl.Biol. Pharm. Technol. 2010; 1: 1168-1174.

Imada, A., Igarasi, S., Nakahama, K. and Isono, M. (1973). L-asparaginase and glutaminase activities of Microorganisms. Journal of General Microbiology, **76:**85-99

Okolo BN., Ezeogu LI., Mba, CN. Production of raw starch digestive amylase by *Aspergillus niger* grown on native starch sources. Journal of Science of Food and Agriculture. 1995; 69, 109–115.

Kapoor M., Nair LM., Kuhad RC. Cost effective xylanase production from free and immobilized *Bacillus pumilus* strain MK001 and its application in saccharification of *Prosopis juliflora*. Biochem.Eng. J. 2008; 38: 88–97.

Pandey A., Soccol CR., RoudriguezLeon JA., Nigam P. In "Soli d State FermentationinBiotechnology Fundamentals and Application s", Asiatech. Publishers. 2001. New Delhi.

Nathiya K., Sooraj SN., Angayarkanni J., Palaniswamy M. Screening of a high glutaminolytic producing strain and its extracellular production by solid state fermentation. Int. J. Pharm. Biosci. 2011; 2: 297-302.

Sivakumar K., Sahu MK., Manivel PR., Kannan L. Optimum co nditions for L-glutaminase production by Actinomycete strain isolated from estuarine fish, Chanos chanos (Forskal, 1775). Indian Journal of Experimental Biology. 2006; 44(3):256-258.

Keerthi TR., Suresh PV., Sabu A., Rajeevkumar S., Chandrasekaran M. Extracellular production of L-glutaminase by alkalophilic *Beauveria bassiana* BTMF S10 isolated from marine sediment. World Journal of Microbiology and Biotechnology. 1999; 15(6):751-752.

Thadikamala Sathish., Reddy Shetty Prakasham. Influence of Fermentation Process Parameters on L-Glutaminase production by *Bacillus subtilis* RSP- International journal of pharmaceutical and chemical sciences GLU. 2012; 1(3**)**: 625-631.

Rajeev Kumar S. and Chanrdasekaran M., Continuous production of L-glutaminase by an immobilized marine *Pseudomonas* sp BTMS-15 in a packed bed reactor., Process Biochemistry, 2003; 38:1431 – 1436.

How to cite this article:

Sunil Dutt P L N S N , Siddalingeshwara K.G, Karthic J, Mohsin S. Mushtaq, Naveen M Vishwanatha T and Prathiba K.S., An Approach on Microbial Biosynthesis of L-Glutaminase – A Tumour Inhibitor. J App Pharm Sci, 2013; 3 (06): 132-135.