Archives of Current Research International

Volume 22, Issue 8, Page 35-45, 2022; Article no.ACRI.94543
ISSN: 2454-7077

Optimization of Process Parameters for L-Glutaminase Production by *Pseudomonas* Species ALG3

J. Okpalla [a*], L. O. Dim [a], D. A. Onyekuru [b], I. E. Duru [a] and T. O. Mba [a]

[a] Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University, P.M.B. 02 Uli, Anambra State, Nigeria.
[b] Department of Science Laboratory Technology, Federal University of Technology, Owerri, Imo State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ACRI/2022/v22i8546

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/94543

Original Research Article

Received: 09/10/2022
Accepted: 16/12/2022
Published: 23/12/2022

ABSTRACT

The present study reports the optimization of process parameters for L-glutaminase production by *Pseudomonas* species ALG3. The L-glutaminase –producing bacterium had already been isolated from soil at a location within Chukwumemeka Odumegwu Ojukwu University, Uli in Anambra state. It was identified using cultural, biochemical and molecular characteristics. Optimization of L-glutaminase production was done by assessing the effect of various parameters using mineral salt medium contained in 250 ml flasks. The parameters included various bivalent metals, pH, surfactants, growth promoters, amino acids and fermentation time. The Results showed that the addition of $FeSO_4$ stimulated optimum L-glutaminase yield of 38.36 U/ml by *Pseudomonas* species ALG3, while the least production was observed with $MnSO_4$. Enhanced L-glutaminase yield of 30.73 U/ml was recorded at pH 8.0, while the lowest enzyme accumulation was observed at pH

**Corresponding author: Email: Judyzuby@yahoo.com, judeokpalla35@gmail.com;*

9.0. The highest L-glutaminase accumulation of 82.25 U/ml was achieved with Tween 80, while the lowest yield was recorded with stearic acid. Urea, stimulated maximum L- Glutaminase yield of 43.82 U/ml, while minimum accumulation was observed with peptone. Proline stimulated highest L-glutaminase yield of 42.63U/ml, while the lowest yield was observed with tyrosine. Highest L-glutaminase yield (91.88 U/ml) was observed after 72h, the yield decreased thereafter. The results obtained in the study illustrated that the optimization of process parameters, increased appreciably the L-glutaminase yield of *Pseudomonas* species ALG3. This suggest that *Pseudomonas* species can be used for large scale production of L- glutaminase, which can offer great potential for applications in Medicine and food industry.

Keywords: L-Glutaminase; Pseudomonas species ALG3; fermentation; bivalent metals; surfactants.

1. INTRODUCTION

"L-Glutaminase (L-glutamine amidohydrolase EC 3.5.1.2) catalyses the hydrolysis of L-glutamine to glutamic acid and ammonia" [1,2,3]. "The enzyme is important in nitrogen metabolism and has a wide distribution in cells of microorganisms, plants and animals" [4]. "Attempts are being made to replace enzymes, which traditionally have been isolated from animal tissues and plants to enzymes from microorganisms" [5,6].

"Several microorganisms which include bacteria, fungi and yeast have been reported to secrete L-glutaminase into fermentation media. There are many numbers of glutaminases reported from different microbial sources with a wide range of fermentation conditions as summarized" by [7].

"The use of microbes as the enzyme producer is more preferable due to their simple growth requirements, easy processing and handling as well as cheaper production" [8]. "L-glutaminase production has been reported from *E. coli* [9], *Bacillus subtilis* [10], *Proteus morganni, P. vulgaris, Xanthomonas juglandis, Erwinia carotovora, E. aroideae, Serratia marcescens, Enterobacter coacae, Klebsiella aerogenes* and *Aerobacter aerogenes"* [11]. "Also, L-glutaminase synthesis has been reported from *Streptomyces rimosus* [12], *Streptomyces* sp.-SBU1 [13] and *Streptomyces avermitilis"* [14].

"Different methods of fermentation technology can be applied for the production of L-glutaminase. Commercial production of L-glutaminase had been carried out using submerged fermentation (SmF) and solid state fermentation (SSF) techniques" [15,16,17]. "L-Glutaminase enzyme has attracted significant attention owing to its potential application in medicine as an anticancer agent, anti-retroviral agent and could be of significance in enzyme therapy of acute lymphocytic leukaemia" [18]. "The enzyme causes selective death of glutamine dependent tumor cells by depriving these cells of glutamine. The use of enzymes to deprive neoplasms of essential nutrients helps in the treatment of malignancies" [19]. "Another most promising application of L- glutaminase is in biosensors for monitoring glutamine levels in mammalian and hybridoma cell cultures without the need of separate measurement of glutamic acid" [20]. "L- Glutaminase is also taking an important role that controls the delicious taste of fermented foods such as soy sauce and in general food products by increasing the glutamic acid content therefore, this enzyme has attracted a great attention in food industries" [21,22].

"Its commercial importance demands the search for new and better yielding microbial strains and economically viable bioprocesses for its large-scale production" [23,24]. "Hence, Researchers are involved in the screening of microbial strains and developing different fermentation strategies to improved productivity. Bioprocess is one of the key processes which helps in enhancing the metabolite productivity under a given set of fermentation environment" [25,26]. "Manipulating the physicochemical parameters of bioprocess plays an imperative role to attain higher metabolite production" [24]. "A variety of the nutritional and incubational parameters have influenced the production of the L-glutaminase enzyme. It is known that the parameters involved in the process of production not only enhance the quantity but also the quality of enzyme, so it becomes more suitable for a specific application. The search for potential microbial strains that hyper produce the enzyme with novel properties for their industrial production is being pursued all over the world" [27].

At present, the requirements of some Nigerian industries for L- glutaminase is met through importation, which involves spending huge

amount of foreign exchange. There is huge potential in the production of L-glutaminase locally by microbiological methods using available raw materials. In our previous study, it was possible to isolate a L- glutaminase producing bacterium *Pseudomonas* species ALG3 from the soil [28]. The present study is a continuation of the research and the aim was to carry out optimization of process parameters for L-glutaminase production by *Pseudomonas* species ALG3.

2. MATERIALS AND METHODS

2.1 Preparation of Inoculum

Two loopfuls (24 h) of *Pseudomonas* species ALG3 were inoculated into 100ml Erlenmeyer flask containing 30ml of seed medium, which was sterilized at 121°C for 15 min. The seed medium was composed of peptone, 10.0g; yeast extract, 10.0g; NaCl, 5.0g; water, 1.0 L; pH adjusted to 7.2. The flasks were incubated for 24 h on a rotary shaker (150 rpm) at 30°C.

2.2 Fermentation Medium

Various 250ml Erlenmeyer flasks containing 50ml of mineral salt glutamine medium that consisted of the following (g/l): glutamine, 10.0; K_2HPO_4, 1.0; KH_2PO_4, 0.1; $MgSO_4$, 1.0; NaCl, 0.5; yeast extract, 0.5, pH 7.0 were used for the experiment.

2.3 Optimization of Process Parameters for L-glutaminase Production

2.3.1 Effect of bivalent metals

The effect of different bivalent metals ($FeSO_4$, $MnSO_4$, combination of $FeSO_4$ +$CuSO_4$, combination of $FeSO_4$ + $MnSO_4$, combination of $CuSO_4$+ $MnSO_4$, combination of $FeSO_4$ + $CuSO_4$ + $MnSO_4$) on L-glutaminase production by *Pseudomonas* species ALG3 was studied. The mineral salt medium as previously described, was supplemented with 0.1%(w/v) of the bivalent metals and sterilized at 121°C for 15 min. Thereafter, the medium was inoculated with 2 ml (4.2×10^6 cfu/ml) of a 24 h seed inoculum. The flasks were placed in a rotary shaker (150 rpm) at 30°C for 72 h. After incubation, 5ml of the fermentation broth was collected and used for the determination of L-glutaminase production and bacteria growth.

2.3.2 Effect of pH

The effect of pH values on L-glutaminase production by *Pseudomonas* species ALG3 was determined. The pH of the mineral salt medium as previously described, was adjusted to various values of 6 to 10 and thereafter sterilized at 121°C for 15 min. Afterward, the flasks containing the medium were inoculated with 2 ml (4.2×10^6 cfu/ml) of a 24 h seed inoculum. The flasks were placed on a rotary shaker (150 rpm) at 30°C for 72 h. After incubation, 5ml of the fermentation broth was collected and used for the determination of L-glutaminase production and bacteria growth.

2.3.3 Effect of surfactant

The effect of surfactants (palmitic acid, stearic acid, oleic acid and tween 80) on L-glutaminase production by *Pseudomonas* species ALG3 was investigated. The mineral salt medium as previously described, was supplemented with different concentrations of surfactants (0.1 to 0.3 % w/v of palmitic and stearic acid and 0.1 to 0.3 % v/v of oleic acid and tween 80) and sterilized at 121°C for 15 min. Afterward, the flasks containing the medium were inoculated with 2 ml (4.2×10^6 cfu/ml) of a 24 h seed inoculum. The flasks were placed on a rotary shaker (150 rpm) at 30°C for 72 h. After incubation, 5ml of the fermentation broth was collected and used for the determination of L-glutaminase production and bacteria growth.

2.3.4 Effect of growth promoters

The effect of different growth promoters (malt extract, beef extract, peptone, tryptone, urea and casein) on L-glutaminase production by *Pseudomonas* species ALG3 was studied. The mineral salt medium as previously described, was supplemented with 0.1% w/v of the different growth promoters and sterilized at 121°C for 15 min. Afterward, the flasks containing the medium were inoculated with 2 ml (4.2×10^6 cfu/ml) of a 24 h seed inoculum. The flasks were placed on a rotary shaker (150 rpm) at 30°C for 72 h. The flasks were placed in a rotary shaker (150 rpm) at 30°C for 72 h. After incubation, 5ml of the fermentation broth was collected and used for the determination of L-glutaminase production and bacteria growth.

2.3.5 Effect of amino acid

The effect of amino acids (glutamic acid, alanine, glycine, threonine, proline and tyrosine) on L-

glutaminase production by *Pseudomonas* species ALG3 was investigated. The mineral salt medium as previously described, was supplemented with 0.1 % (w/v) of different amino acids and sterilized at 121°C for 15 min. Afterward, the flasks containing the medium were inoculated with 2 ml (4.2×10^6 cfu/ml) of a 24 h seed inoculum. The flasks were placed on a rotary shaker (150rpm) at 30°C for 72 h. After incubation, 5ml of the fermentation broth was collected and used for the determination of L-glutaminase production and bacteria growth.

2.3.6 Effect of fermentation time

The effect of fermentation time on growth and L-glutaminase production by *Pseudomonas* species ALG3 was studied. The mineral salt medium as previously described, was sterilized at 121°C for 15 min and thereafter, inoculated with 2 ml (4.2×10^6 cfu/ml) of a 24 h seed inoculum. The flasks containing the medium were placed on a rotary shaker (150rpm) at 30°C for 168 h. At interval of 24 h, 5ml of the fermentation broth was collected and used for the determination of b acteria growth, pH and L-glutaminase production.

2.4 Estimation L-glutaminase Activity

L-glutaminase was assayed according to the method described by [16]. An aliquot of 0.5 ml of the sample was mixed with 0.5 ml of 0.04 ML-glutamine solution in the presence of 0.5 ml of distilled water and 0.5 ml of phosphate buffer (0.1 M, pH 8). The mixture was incubated at 37°C for 30 min and the reaction was arrested by the addition of 0.5 ml of 1.5 M trichloroacetic acid. From this mixture, 0.1 ml of the mixture was taken out and mixed with 3.7 ml of distilled water and 0.2 ml of Nessler's reagent and A450 nm was detected. Activity of the enzyme was determined in Unit/ml (U/ml) and L-glutaminase unit is the amount of the enzyme that liberates one µMol of ammonia.

2.5 Determination of Growth

It was turbidmetrically determined from the culture broth in spectrophotometer at 660 nm.

2.6 Statistical Analysis

The data obtained were analyzed by covariance matrix analysis using Microsoft excel 2013.

3. RESULTS

The result of the effect of bivalent metals on L-glutaminase production by *Pseudomonas* species ALG3 is shown in Fig. 1. The result showed that maximum L-glutaminase yield (38.36 U/ml) was achieved with $FeSO_4$, while the least was recorded in $MnSO_4$. The bacteria growth was highest with the combination of $FeSO_4$ and $CuSO_4$ and lowest with lowest with $CuSO_4$.

The result of the effect of pH on L-glutaminase production by *Pseudomonas* species ALG3 is shown in Fig. 2. The result showed that highest L- glutaminase yield (30.73 U/ml) was observed at pH of 8.0, while the lowest was recorded at pH of 9. Maximum bacteria growth was observed at pH of 7, while the lowest was observed at pH of 9 and 10. The covariance matrix analysis showed that there was a significant high value of effect at pH of 8.0 for L-glutaminase production.

The result of the effect of surfactants on L-glutaminase production by *Pseudomonas* species ALG3 is shown in Fig. 3. The result showed that highest L-glutaminase yield (82.25 U/ml) was achieved at 0.2% (v/v) Tween 80, while the least was observed at 0.2% (w/v) stearic acid. The bacteria growth was highest at 0.3% (v/v) oleic and lowest with 0.3% (w/v) palmitic acid.

The result of the effect of growth promoters on L-glutaminase production by *Pseudomonas* species ALG3 is shown in Fig. 4. The result showed that the highest L-glutaminase yield (43.82 U/ml) was observed in urea, while the least was recorded in peptone. Maximum bacteria growth was observed in peptone, while the lowest was recorded in urea. The covariance matrix analysis shows that there was a significant high value of effect in urea for L- glutaminase production.

The result of the effect of amino acids on L-glutaminase production by *Pseudomonas* species ALG3 is shown in Fig. 5. The result showed that highest L- glutaminase yield (42.63U/ml) was observed with proline, while the least was recorded with tyrosine. The bacteria growth was also highest with proline and lowest with threonine.

The result of the effect of fermentation time on growth and L-glutaminase production by *Pseudomonas* species ALG3 is shown in

Fig. 6. The result shows that highest L-glutaminase yield (91.88 U/ml) was observed in 72h, while the least was recorded in 144 h. *Pseudomonas* growth was also highest within 72h and lowest within 96h.

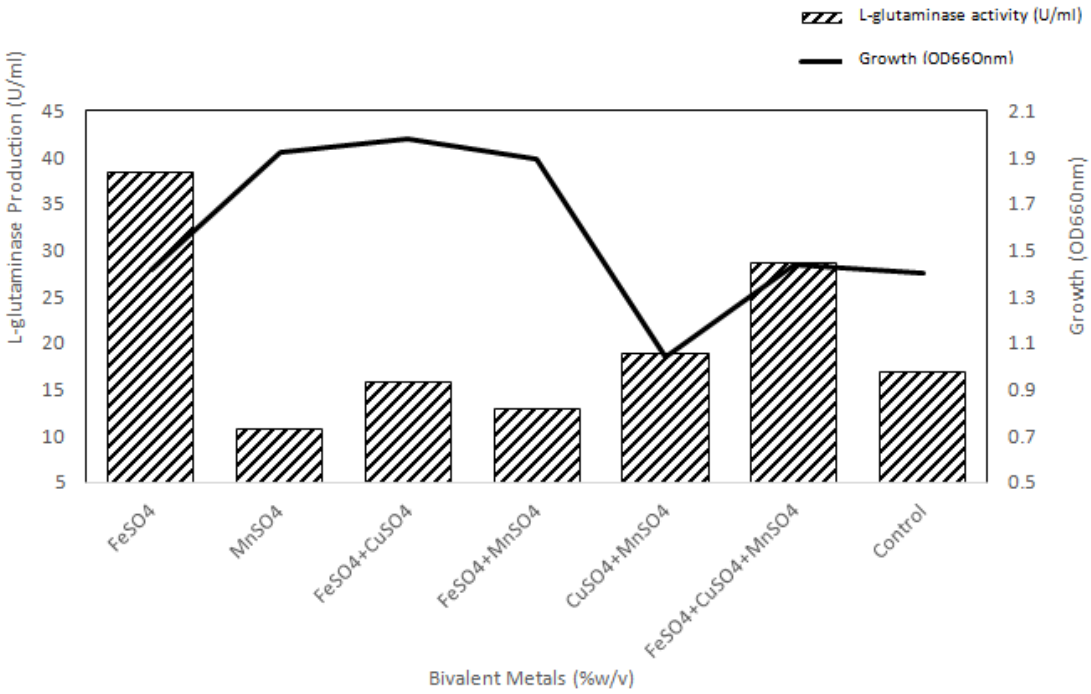

Fig. 1. The effect of bivalent metals on L-glutaminase production by *Pseudomonas* species ALG3

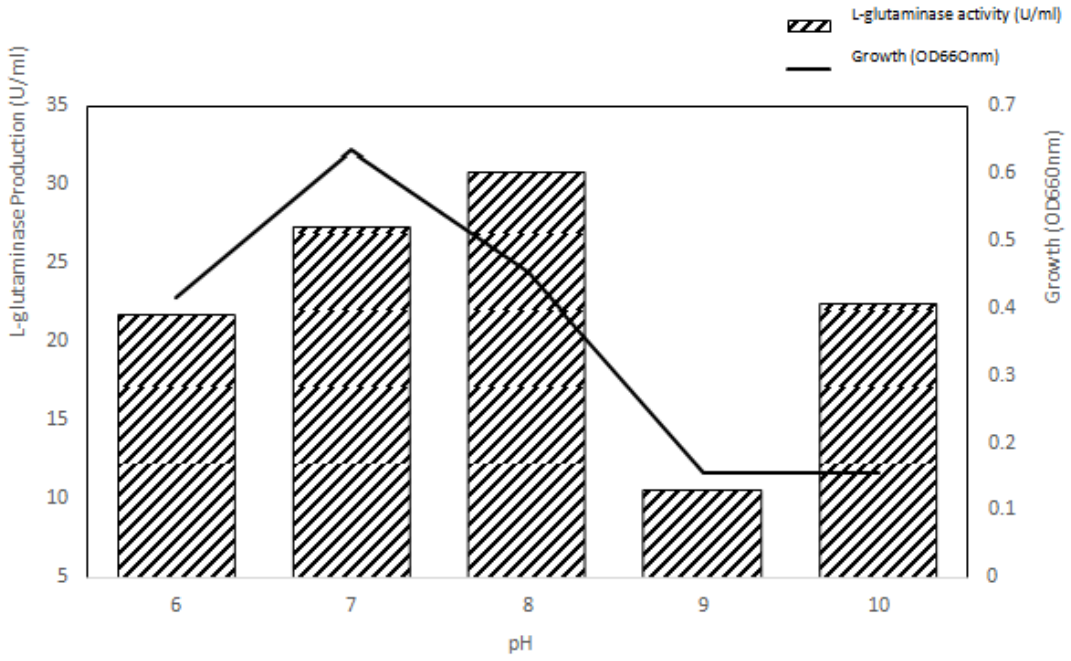

Fig. 2. The effect of pH on L-glutaminase production by *Pseudomonas* species ALG3

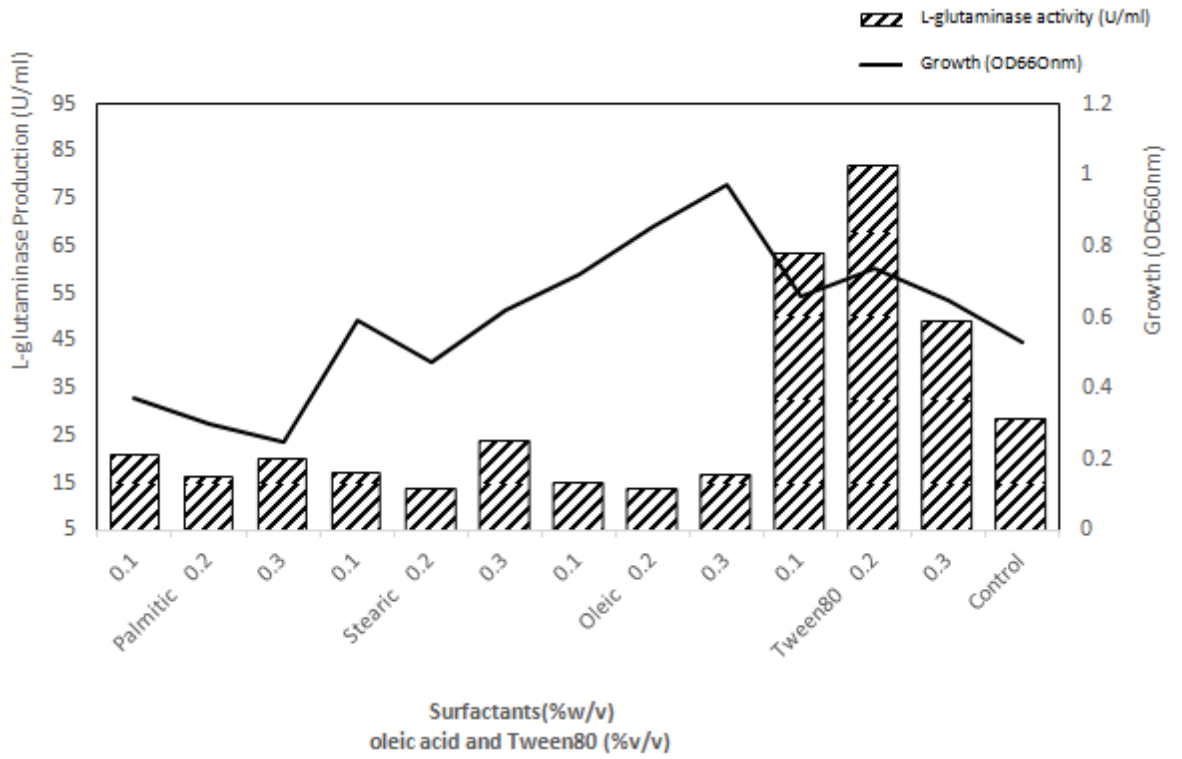

Fig. 3. The effect of surfactants on L-glutaminase production by *Pseudomonas* species ALG3

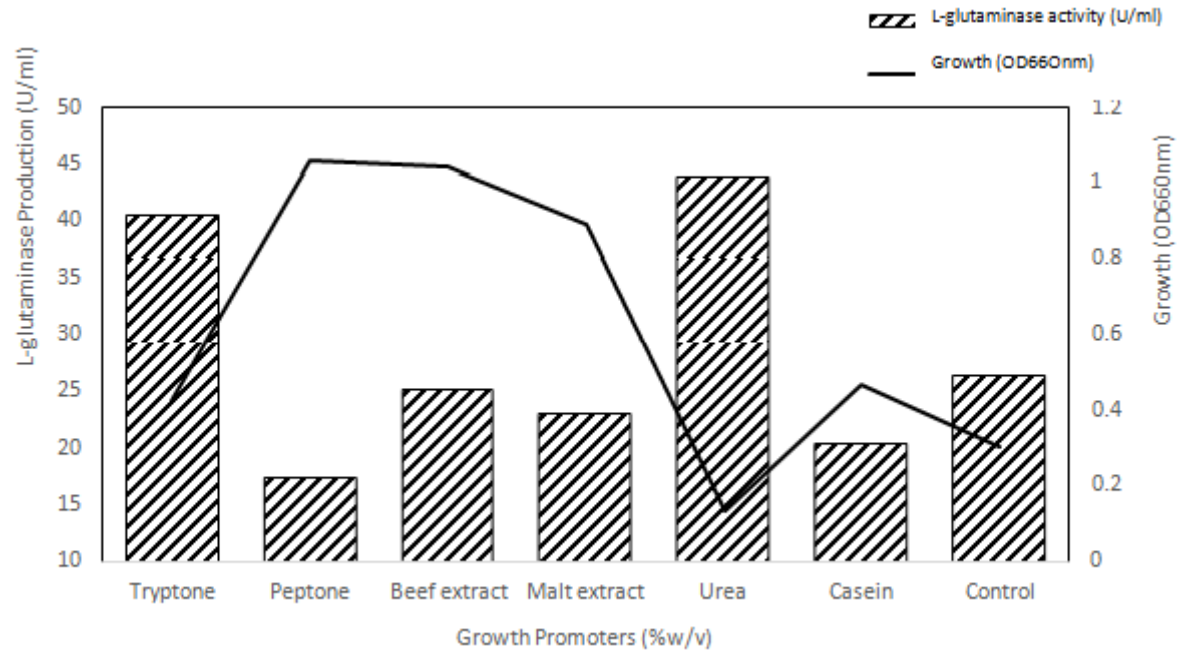

Fig. 4. The effect of growth promoters on L-glutaminase production by *Pseudomonas* species ALG3

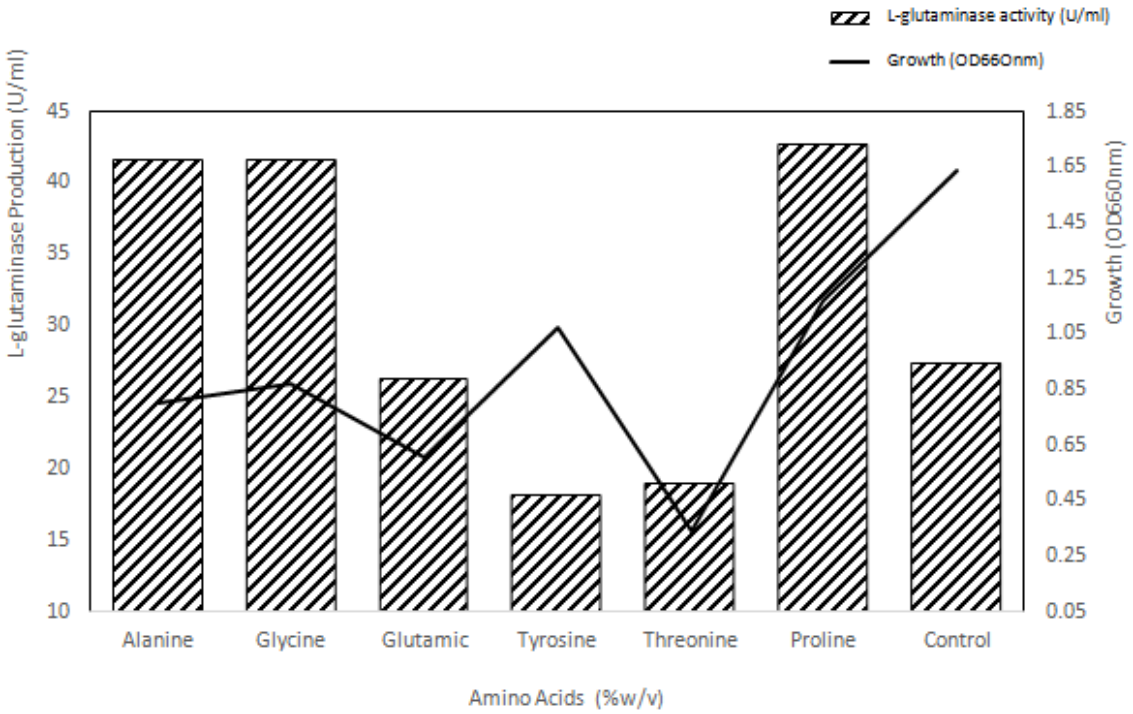

Fig. 5. The effect of amino acids on L-glutaminase production by *Pseudomonas* species ALG3

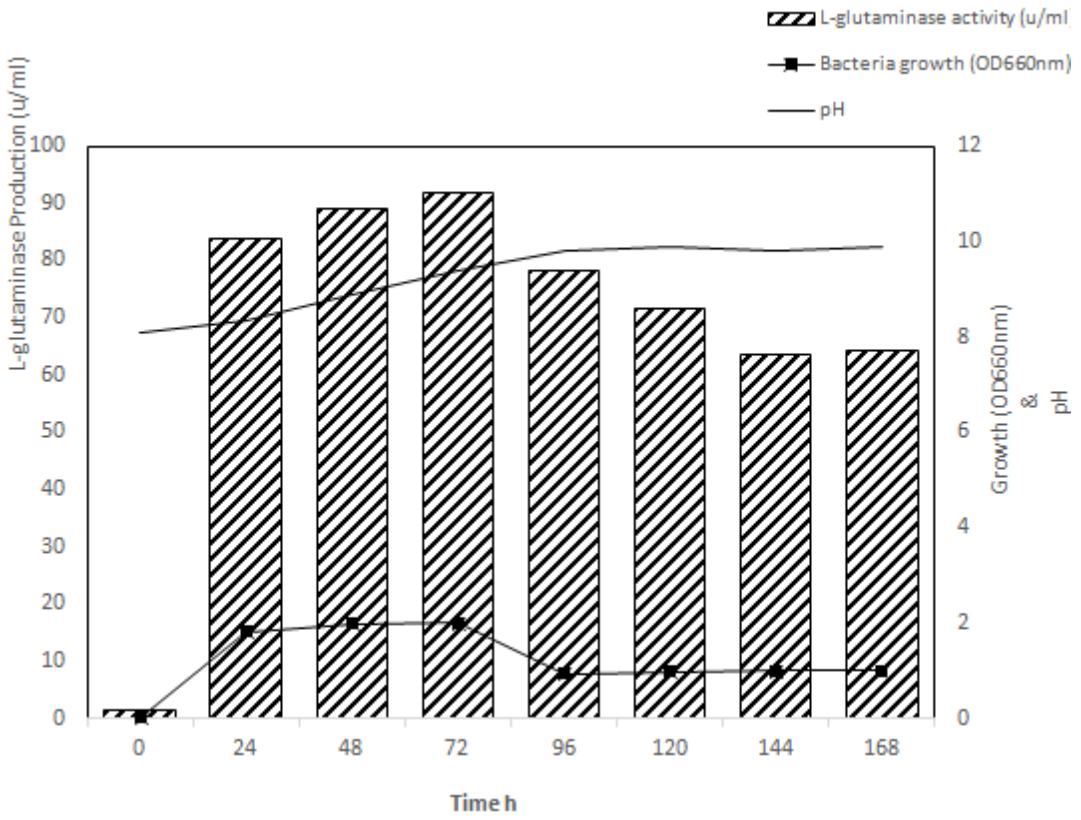

Fig. 6. The Effect of Fermentation Time on L-glutaminase production by *Pseudomonas* species ALG3

4. DISCUSSION

The result obtained from this study indicated that the addition of $FeSO_4$ to the medium generated the highest production of L-glutaminase. This contradicted the report of [29], who studied "the effect of four bivalent metal ions (Mg^{2+}, Mn^{2+}, Zn^{2+} and Fe^{2+}) supplementation on L-glutaminase yield from three bacteria isolates and found only Mg^{2+} and Mn^{2+} ions to slightly improve enzyme yield, while both Zn^{2+} and Fe^{2+} ions had negative impact on enzyme yield". Metal ions play a vital role in fermentation as they are co-factors for various enzymes and also facilitate the transport of materials across cell membrane. Martin and McDaniel [30], Hughes and Poole [31] highlighted that the metal ions probably acted as activators or inhibitors of enzymes involved in the synthetic steps of metabolites. It has been reported that Fe^{2+} and Mn^{2+} are the most important of the trace elements as they play a role in the excretion of primary metabolites.

It was observed that at pH 8 the amount of L-glutaminase produced was highest. recorded in the study. This is not in accordance with the reports of [9,15,32,18], who observed maximum L-glutaminase production at pH 7 for *Streptomyces* sp, *Trichoderma koningii, Bacillus* species and *Vibrio costicola* respectively. [33,34,35] observed that some microbial species were known to produce L-glutaminase in the neutral or slightly alkaline pH under submerged fermentation conditions. In other reports, L-glutaminase production at pH 6.0 was reported in *Cryptococcus nodaensis* [36] and *Pseudomonas* sp. [37]. Nathiya et al. [38], observed higher L-glutaminase production by *Aspergillus flavus* at acidic pH 4, while the activity decreased up to 50% at neutral pH 7. Abdallah, et al. [39], reported that the optimum pH was 7.0 to 8.0 for *Streptomyces avemitilis*.

The surfactant Tween 80, was observed to stimulate maximum L-glutaminase production in the work. Surfactants decrease surface tension and increase the air supply of the medium. Various kinds of surface active agents are known to affect permeability in microorganisms [40,41,42], revealed that addition of Tween 80 increased yield of enzymes like cellulase, amylase and sucrase.

In the study the amino acid proline stimulated increased production of L- glutaminase enzyme. This finding is contrary to reports of other workers. Kiruthika, et al. [43], observed that "among the different amino acids, L-glutamine which is the actual substrate for L-glutaminase showed a significant increase in the production of the enzyme". Another observation was made by [12] who reported "maximal L-glutaminase production at 2% L-glutamine in wheat bran". However, Renu [44], has reported that "L-glutamic acid and lysine at 1% (w/v) were found to induce maximum L-glutaminase production in *V. cholera* ACMR 347 and *P. fluorescens* ACMR 171". [45], observed that among the amino acids utilized 0.1% cysteine supported the highest L-glutaminase production of 135.98 U/ml.

The fermentation time of the modified fermentation medium indicated maximum yield at 72h and further increase in incubation period showed low yield of L-glutaminase. This is in line with the report of Rashmi, et al. [46] who observed maximum yield at 72 h of fermentation in optimization of submerged fermentation process for L-glutaminase production by *Pseudomonas aeruginosa* BGNAS-5.

5. CONCLUSION

The Optimization studies conducted on *Pseudomonas* species ALG3 showed that the supplementation of $FeSO_4$, Tween 80, urea, proline and beef extract stimulated improved L-glutaminase yield. Also, the pH 8 encouraged optimum L- glutaminase production, while the optimum fermentation time was observed at 72h. Comparatively, fermentation time of 72h, accumulated the highest glutaminase yield of 91.88 U/ml, followed by the addition of Tween 80 which yielded 82.25 U/ml. This indicated that *Pseudomonas* species ALG3, which was isolated from the soil has immense potential as an industrial organism for the production of L-glutaminase using submerged fermentation. Thus, this will ensure its availability, low cost and less dependence on importation. Further research is needed to study the effects of other parameters on optimum glutaminase accumulation by *Pseudomonas* species.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ohshima M, Yamamoto T, Soda K. Effect of glutamine analogs on glutaminase formation in *Pseudomonas aeruginosa*.

Bulletin Institute for Chemical Research Kyoto University. 1976;54:170-175.
2. Iyer P, Singhal RS. Glutaminase production using *Zygosaccharomyces rouxii* NRRL-Y 2547: Effect of aeration, agitation regimes and feeding strategies. Chem Eng Technol. 2010;33:52-62.
3. Krishnakumar S, Alexis Rajan R. Ravikumar S. Extracellular production of L-glutaminase by alkalophilic *Streptomyces sp*.- SBU1 isolated from Cape Comorin coast. Indian J. Geo Mar. Sci. 2011;40(5):717-721.
4. El-Ghonemy DH. Microbial amidases and their industrial applications: A review. J Med Microbiol Dia. 2015;4:173.
5. Teja DD, Devi VS, Harsha N, Vishala SS, Lakshmi PK. Production of L-glutaminase from marine ecosystems and optimal conditions for maximal production by actinomycetes. Int J Adv Res. 2014;2(1): 485–491.
6. Aishwariyaa LV, Sheeja L, Devi MS. Screening and identification of asparginase and glutaminase producing halophilic bacteria from natural saline habitats. Int J Rec Adv Biotechnol Nanotechnol. 2020;3:34–53.
7. Nandakumar R, Yoshimune K, Wakayama M, Moriguchi, M. Microbial glutaminase: biochemistry, molecular approaches and applications in the food industry. J Mol Cataly. 2003;23(2-6):87-100.
8. Katikala PK, Varaprasad B, Tadimalla P, Guntuku GS. Screening of L-glutaminase producing marine bacterial cultures for extracellular production of L-glutaminase. Internat J. Chem Tech Res. 2009;1(4): 1232-1235.
9. Prabhu GN, Chandrasekaran M. (1997). Impact of process parameters on L-glutaminase production by marine *Vibrio costicola* in solid state fermentation using polystyrene as an inert support. Proc Biochem.1997;32:285-289.
10. Dubey R, Paul A, Prity N. Isolation, production and screening of anti-cancer enzyme L-glutaminase from *Bacillus subtilis*. Internat J Pharma Bio Sci. 2015;5: 96-105.
11. Wade HE, Robinson HK, Phillips BW. Asparaginase and glutaminase activities of bacteria. J Gen Microbiol. 1971;69:299-231.
12. 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 J Microbiol Biotech. 1999;15:751-752.
13. Krishnakumar S, Rajan RA, Ravikumar S. Extracellular production of L-Glutaminase by marine alkalophilic *Streptomyces sp*.-SBU1 isolated from Cape Comorin coast, Ind J Geo-Mar Sci 2011;40:717-721.
14. Abdallah NA, Amer SK, Habeeb MK. Screening of LGlutaminase produced by actinomycetes isolated from different soils in Egypt. Internat J ChemTech Res. 2012;4: 1451-1460.
15. El-Sayed ASA. L-glutaminase production by *Trichoderma koningii* under solid state fermentation. Ind J Microbiol. 2009;49: 243–250.
16. Imada A, Igarasi S, Nakahama K, Isono M. Asparaginase and glutaminase activities of micro-organisms. J. Gen. Microbiol. 1973;76:85-99.
17. Yamamoto S, Hirooka H. Production of glutaminase by *Aspergillus sojae*. J. Ferment. Technol. 1974;52:564-569.
18. Savitha SD, Sonal JC, Basavaraj SH. Production, purification and characterization of L-glutaminase from *Streptomyces* sp. isolated from soil. J Appli Pharma Sci. 2016;6(7):100-105.
19. Schmid FA, Roberts J. Antineoplastic and toxic effects of *Acinetobacter* and *Pseudomonas* glutaminase - asparaginases. Cancer Chemo Rep. 1974;58:829-840.
20. Sabu A, Keerthi TR, Kumar SR, Chandrasekaran M. L-Glutaminase production by marine *Beauveria* sp. under solid state fermentation. Proc Biochem. 2000;35:705–710.
21. Moriguchi M, Sakai K, Tateyama R, Furuta Y, Wakayama M. Isolation and characterisation of salt tolerant glutaminase from marine *Micrococcus luteus* K-3. J. Ferm. and Bioeng. 1994; 77:621-625.
22. Alexandra WZ, Christiane GD, Affolter M. Functional characterization of a Salt-and thermotolerant glutaminase from *Lactobacillus rhamnosus*. Enz Micro Technol. 2003;32:862-867.
23. Nagendra PG, Chandrasekaran M. Impact of process parameters on L-glutaminase production by marine *Vibrio costicola* in solid state fermentation using polystyrene as an inert support. Proc Biochem. 1997; 32:285–289.

24. Binod P, Sindhu R, Madhavan A, Abraham A, Mathew AK, Beevi US, Sukumaran RK, Singh SP, Pandey A. Recent developments in L-glutaminase production and applications - An overview. Biores Technol. 2017;245:1766-1774.
25. Park Y, Kang S, Lee J, Hong I, Kim W. Xylanase production in solid state fermentation by *Aspergillus niger* mutant using statistical experimental designs. Appl. Microbiol. Biotechnol. 2002;58:761–766.
26. Prakasham RS, Rao ChS, Rao RS, Lakshmi GS, Sarma PN. L-asparaginase production by isolated *Staphylococcus sp*. – 6A: design of experiment considering interaction effect for process parameter optimization. J Appl Microbiol. 2007a;102: 1382–1391.
27. Prabhu GN, Chandrasekaran M. Polystyrene - an inert carrier for glutaminase production by marine *Vibrio costicola* under solid-state fermentation. World J Microbiol Biotech, 1995;11:683-684.
28. Okpalla J, Umeh SO, Dim OL. L-glutaminase production by a terrestrial *Pseudomonas* species isolated from Nigerian soil. Internat J Agri Biosci. 2019; 8(2):62-66.
29. Jesuraj SA, Praya J, Kingsley R, Kumar LD, Ravikumar M. Bioprocessing of Ar isolates for economical production of l - glutaminase by solid state fermentation. International Journal of Chem. Tech. Res. 2013;5(4):1428-1436.
30. Martin JE, McDaniel IE. (1977). Production of polyene macroalide antibiotics. Adv Appl Microbiol.1977;21:1-52.
31. Hughes MN, Poole RK. Metal and microorganism. Chapman and Hall, USA. 1989;1-37.
32. Sinha S, Nigam VK. Production and characterization of L- glutaminase by *Bacillus* sp. Internat J Pharma Sci Res. 2016;7(4):1620-26.
33. Roberts J, Holcenberg JS, Dolowy WC. Isolation, crystallization, and properties of *Achromobacteriaceae* Glutaminase-asparaginase with antitumor activity. J Biol Chem. 1972;247:81-90.
34. Wakayama M, Yamagata T, Kamemura A, Bootim N, Yano S, Tachiki T, Yoshimune K, Moriguchi M. Characterization of salt-tolerant glutaminase from *Stenotrophomonas maltophilia* NYW-8 1 and its application in Japanese soy sauce fermentation. J Ind Microbiol Biotech 2005;32:383–390.
35. Iyer P, Singhal RS. Production of glutaminase (E.C.3.2. 1.5) from *Zygosaccharomyces rouxii*: Statistical optimization using response surface methodology. Biores Technol. 2008;99: 4300-4307.
36. Sato I, Kobayashi H, Hanya Y, Abe1 K, Murakami S, Scorzetti G, Fell JW. *Cryptococcus nodaensis* sp nov, a yeast isolated from soil in Japan that produces a salt-tolerant and thermostable glutaminase. J Ind Microbiol Biotech. 1999;22:127-132.
37. Kumar SR, Chandrasekaran M. Continuous production of Lglutaminase by an immobilized marine *Pseudomonas* sp. BTMS51 in a packed bed reactor. Pro Biochem 2003;38:1431-1436.
38. Nathiya K, Nath SS, Angayakanni J, Palaniswamy M. Optimised production of L-glutaminase: A tumor inhibitor from *Aspergillus flavus* cultures on agroindustrial residues. Afri J Biotech. 2011;10:13887-13894.
39. Abdallah NA, Amer SK, Habeeb MK. Production, purification and characterization of L-glutaminase enzyme from *Streptomyces avermitilis*. Afri J Microbiol Res. 2013;7:1184-1190.
40. Oshima J, Tanaka K, Kinoshita S. Studies on L-glutamic acid fermentation xiii. Effect of surface-active agent on the conversion of citrate to L-glutamate with resting cells of *Micrococcus glutamicus*. J Gen Appl Microbiol. 1964;10:333-342.
41. Rehacek Z, Basappa SC. (1971). Effect of tween 80 on alkaloid producing cultures of *Claviceps paspali.* Folia Microbiol. 1971; 16:110.
42. Reese ET, Maguire A, Parrish FW. Glycosides and exo-glucanase, Canad J Biochem. 1968;46:25-33.
43. Kiruthika J, Saraswathy N, Murugesan S. Maximizing L-glutaminase production from marine *Bacillus subtilis* JK-79 under solid state fermentation. Afr. J. Biotechnol. 2018;17(9):288-305.
44. Renu S. (1991). L-glutaminase production by marine Bacteria, Ph.D thesis, Cochin University of Science and Technology, Cochin, India; 1991.
45. Ahmed MMA, Taha TM, Abo-Dahab NF, Hassan FSM Process Optimization of L-glutaminase Production; A tumour inhibitor

from marine endophytic isolate *Aspergillus sp*. ALAA-2000. J Microb Biochem Technol. 2016;8:382-389.

46. Rashmi AM, Gopinath SM, Krishan K, Narasimha MTP. Optimization of submerged fermentation process for l-glutaminase produced *Pseudomonas aeruginosa* BGNAS-5. Internat J lates Res Sci and Technol. 2012;1(4):308-310.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/94543