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ISOLATION AND CHARACTERISATION OF L-GLUTAMINASE PRODUCING FUNGI FROM MARINE SOURCE

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Keywords:

Estuaries region, Marine *Aspergillus flavus* JK-79, L-glutaminase submerged fermentation, OFAT

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ABSTRACT: L-glutaminase [EC.3.5.1.2] is an amidohydrolase that catalyzes the hydrolytic deamination of L-glutamine, resulting in the production of L-glutamic acid and ammonia. The L-glutaminase has received a significant attention due to its potential as an anticancer agent. In the present study, soil sediments were collected from the estuaries region of Parangipettai, Cuddallore District, Tamil Nadu. The isolated fungal strains were screened for L-glutaminase production, and the potent strain was characterized and identified as *Aspergillus flavus* JK-79 (Genebank accession number MF445235). The anti-cancer enzyme L-glutaminase production by *Aspergillus flavus* JK-79 was evaluated in five different production media under the submerged fermentation process. The Minimal medium showed maximal enzyme production by the marine isolate. Further, parameters such as Inoculum size, pH, temperature, and nutritional requirements such as carbon sources, nitrogen sources, and amino acids were optimized by One-factor-at-a-time (OFAT) for maximal production of L-glutaminase under submerged fermentation process. The maximum production of L-glutaminase by *Aspergillus flavus* JK-79 was recorded at pH 8 (790 .436U/mL), temperature 28°C with 2% inoculum size. The findings suggest that the enzyme L-glutaminase from marine *Aspergillus flavus* JK-79 may be suitable for therapeutic application in the treatment of ALL (Acute Lymphoblastic Leukemia) with less side effects. Further, the marine strain can also be promising for industrial-scale production due to its enhanced level of L-glutaminase productivity.

INTRODUCTION: Enzymes are specialized proteins that cooperate with vitamins and minerals and act as a catalyst to accelerate the chemical reaction in biological cells. Enzymes are highly specific in their action towards the substrate, and often, many different enzymes are required to bring about by concerted actions [1].

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Enzymes are in great demand for use in pharmaceuticals. The manufacturing or processing of enzymes for use as a drug is an important fact of today's pharmaceutical industry. Attempts to capitalize on the advantage of enzymes as drugs are now being made at virtually every pharmaceutical research center in the world.

Enzyme supplements are available in pills, capsules, and powders that often combine several enzymes. Microbial enzymes are preferred over plant or animal sources due to their economic production, consistency, process modification, and optimization.

They are relatively more stable than the corresponding enzymes derived from plants or animals [2, 3, 46]. Cancer, particularly Acute Lymphoblastic Leukaemia (ALL), is a global problem. Despite sincere efforts paid in the past, the search for efficient drugs to solve this problem is being continued worldwide. Unlike normal cells, leukemic cells do not depend on L-glutamine synthetase; they directly depend on the exogenous supply of L-glutamine from the blood for their growth and survival [4, 44, 45]. Therefore, blood L-glutamine serves as a metabolic precursor for the nucleotide and protein synthesis of tumor cells. Consequently, L-glutaminase causes selective death to L-glutamine-dependent tumor cells by blocking the energy route for their proliferation [5, 6, 7]. The ability of the enzyme to bring about degradation of glutamine poses it as a possible candidate for enzyme therapy, which may soon replace or combined with L-asparaginase in the treatment of acute lymphocytic leukemia; it is found that administration of L-glutaminase will deplete L-glutamine, which is required for asparagine synthesis in the body of patient thereby inhibiting asparagine dependent protein synthesis and eventually the synthesis of DNA and RNA [8, 9, 10]. Although several kinds of treatments are available, enzyme therapy is equally effective. L-asparaginase and L-glutaminase (L-glutamine amidohydrolase EC 3.5.1.2.) earned attention since the discovery of their antitumor properties of economic importance from potential marine microorganisms, especially fungi [11]. L-glutaminase (EC 3.5.1.2) is an amidohydrolase that catalyzes the hydrolytic deamination of L-glutamine, resulting in the production of L-Glutamic acid and ammonia [12] **Fig. 1**. L-glutaminase is ubiquitous in the biological world, and organisms ranging from bacteria to human beings have the enzyme. A variety of microorganisms, including bacteria, yeast, molds, and filamentous fungi, have been reported to produce L-glutaminase, of which the most potent producers are fungi [13, 14, 15]. L-glutaminase is the cellular enzyme that deaminates the L-glutamine. The action of glutaminase plays a major role in the cellular nitrogen metabolism of both prokaryotes and eukaryotes. L-glutaminase received much attention with respect to the therapeutic and industrial applications [16].

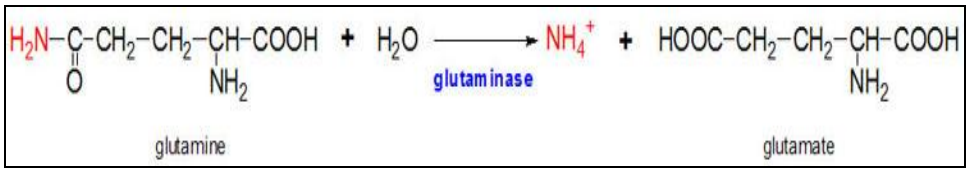


FIG. 1: HYDROLYSIS OF L-GLUTAMINE TO L-GLUTAMATE BY L-GLUTAMINASE

The Marine biosphere is one of the richest habitats, which is less explored. Because of the diversity and scale offer enormous opportunities for non-destructive exploitation within many facets of modern biotechnology [17]. Knowledge of marine microbes is limited, and they remain untapped sources of many metabolites with novel properties [18]. Marine microbes and their enzymes are industrially important due to their increased tolerance to salinity and are being intensively screened for products of economic importance [19]. Further, as of date, marine microorganisms remain as untapped sources of many metabolites with novel properties. Marine microorganisms have a diverse range of enzymatic activity and are capable of catalyzing various biochemical reactions with novel enzymes. Thus, there is enormous scope for the investigations exploring the probabilities of deriving new products.

Moreover, the marine environment, particularly seawater, which is saline in nature and chemically closer to human blood plasma, could provide safer microbial enzymes with no or less side effects when administered for human therapeutic application. The major restriction in the application of terrestrial L-asparaginase in the treatment of ALL is the development of allergic reactions in the patients and ultimately leading to inactivation of the drug [20]. This could be effectively suppressed by the application of L-glutaminase from marine source [21]. With this idea, the present work is intended to isolate and characterize a potent marine fungal strain capable of producing the L-glutaminase enzyme. Further, the physical and nutritional parameters were optimized to enhance the L-glutaminase production by one factor at a time method (OFAT) [22, 23, 24].

MATERIALS AND METHOD:

Source of Strain: Soil samples were collected from different seashores of Parangipettai (Lat. 11° 42' N; Long. 79° 46' E) in sterile polythene bags and transported to the laboratory. The samples were stored at 4°C till further use.

Enrichment and Selective Isolation of L-Glutaminase Isolates: One gram of soil sample was suspended in 100 ml of seawater and placed in an incubator (28°C) for 30 min for the complete mixing of the soil sediment. About 1.0 ml of the supernatant solution was serially diluted in sterile seawater and 0.1 ml of this was spread on Minimal Glutamine Agar (MGA) medium (0.5 KCl; 0.5 $MgSO_4$; KH_2PO_4; 0.1 $FeSO_4$; 0.1 $ZnSO_4$; 10 glutamine, 0.12 phenol red (g/l). L-glutamine act as the sole carbon and nitrogen source, red phenol act as a pH indicator.

The plates were incubated at 28°C for 5 days [25, 26]. All the media in the present work was prepared with seawater which has 30 ppt (parts per thousand) of salinity. Isolated fungal strains were maintained in potato dextrose agar (PDA) medium [27] and the cultural characterization was performed.

Determination of L-Glutaminase Activity: L-glutaminase activity was measured by the Imada *et al.* method (1973) [28, 44]. The different isolates were grown for 5 days at 28°C and harvested by centrifugation at 10,000 rpm for 20 minutes, and the supernatant was used for L-glutaminase assay. An aliquot of 0.5 ml of the sample was mixed with 0.5 ml of 0.04 M L-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 trichloro acetic acid. To 0.1 ml of the mixture, 3.7 ml of distilled water and 0.2 ml of Nessler's reagent were added. The absorbance was measured at 450nm using a UV-Visible spectrophotometer. One international unit of L-glutaminase was defined as the amount of enzyme that liberates one µ mole of ammonia under optimal conditions. The enzyme yield was expressed as Units/ml (U/ml of culture supernatant) [17].

Staining of Fungi with Lactophenol Cotton Blue: Lactophenol Cotton Blue Staining was performed, and the preparation was examined under a microscope with required magnification for the presence of characteristic mycelia [29, 47].

Molecular Characterization of Potent Fungal Strain: Molecular characterization of the selected fungal isolate was done at IMTECH Chandigarh INDIA by using intergenic transcribed spacer (ITS)/5.8S rRNA gene sequence analysis. The sequence obtained was deposited at GenBank (NCBI). The evolutionary history was inferred using the Neighbor-Joining method [30, 49].

Production Medium: Five different types of production media were evaluated for the production of L-glutaminase enzyme by submerged fermentation (SmF).

Production Medium	Composition (g/L)
Medium 1Czapek Dox's Medium [27, 31,48]	L-glutamine -10, Glucose-5, KH_2PO_4 - 1.5, $MgSO_4$. $7H_2O$ - 0.5, KCl-0.5 $CuNO_3.7H_2O$ - 0.02, $ZnSO_4.7H_2O$ - 0.02, $FeSO_4$. $7H_2O$ - 0.02 Aged sea water – 1000 ml (30 ppt of salinity)
Medium 2 Czapek Dox's Medium (Modified) [32]	Glucose – 2, L-glutamine – 10, KH_2PO_4 - 1.5, $FeSO_4$. $7H_2O$- 0.01, $MgSO_4$. $7H_2O$ - 0.52, KCl - 0.52, Aged sea water – 1000 ml (30 ppt of salinity)
Medium 3 Mineral Salt Glutamine (MSG) [33, 34]	KH_2PO_4 - 0.1, $MgSO_4$. $7H_2O$ - 0.5, $CaCl_2.2H_2O$ - 0.1, $NaNO_3$ - 0.1, $FeCl_3$ - 0.1 L-glutamine – 10, NaCl – 10, Mineral water - 1000ml
Medium 4 Minimal Medium [35]	KH_2PO_4 – 1, $MgSO_4$. $7H_2O$ - 0.5, L-glutamine – 10, $FeSO_4$. $7H_2O$ - 0.1 $ZnSO_4.7H_2O$ - 0.1, KCl - 0.5, Aged sea water – 1000 ml (30 ppt of salinity)
Medium 5 M9 Medium [24,36]	$Na_2HPO_4.H_2O$ – 6, KH_2PO_4 – 3, L-glutamine - 5, $MgSO_4$. $7H_2O$ - 0.5 $CaCl_2.2H_2O$ - 0.002, Glucose – 5, Aged sea water – 1000 ml (30 ppt of salinity)

Optimization of Process Parameters for L-glutaminase Production by Marine Fungi: Various physiochemical parameters and media components were tested as a single variable by OFAT approach [22, 45]. The effect of individual parameters such as pH, temperature and inoculum size were investigated on the production of L-glutaminase. Following this, the effect of different carbon sources, nitrogen sources and amino acids were also analyzed. The independent parameters were evaluated keeping other parameters at constant level and selected parameters were

incorporated in the next experiment while optimizing the next parameter. All the fermentation experiments were carried out in triplicates in 250 ml Erlenmeyer flasks.

Effect of Temperature: The optimum temperature required for maximal L-glutaminase production by the fungal strain under submerged fermentation was estimated by incubating the inoculated Minimal medium at various temperatures (28 - 50^{o}C) in a shaker for 5 days. After incubation, the fermented broths were centrifuged, supernatant was collected, and the yield of L-glutaminase was estimated.

Effect of Inoculum size: The Minimal media was inoculated at various inoculum concentrations of 1-6% and incubated at 28^{o}C for 5 days. The yield of L-glutaminase was determined.

Effect of pH: The strain was inoculated in 100ml of SWG media, which was prepared in various pH of 5 to 9 and incubated in at 28^{o}C for 5 days. The yield of L-glutaminase was measured by Imada et al. 1973 method [28].

Effect of Additional Carbon Sources: Adding other carbon sources along with glutamine was analyzed by incorporating (Mannitol, sucrose, sorbitol, glucose, maltose, and Mannose) in the medium individually at 1% (w/v) level. The yield of L-glutaminase was determined.

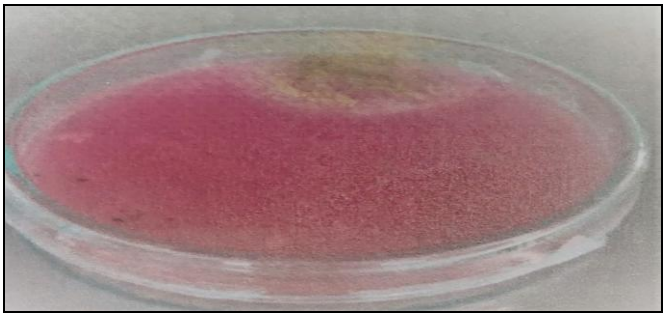
Effect of Additional Nitrogen Sources: The effect of adding other nitrogen sources along with glutamine was analyzed by incorporating (Peptone, Yeast extract, Ammonium sulphate, Malt extract, Ammonium nitrate, and Potassium nitrate) in the medium individually at 1% (w/v) level. The yield of L-glutaminase was determined.

Effect of Amino Acids: The effect of adding other amino acids on the production of L-glutaminase enzyme was analyzed by incorporating (L-glutamic acid, L-asparagine, Arginine, Methionine, and Proline) in the medium individually at 1% (w/v) level. The yield of L-glutaminase was determined.

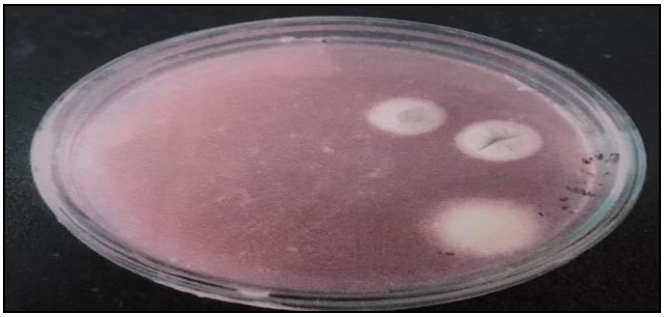
RESULTS AND DISCUSSION:

Screening of L-glutaminase Producing Fungal Strain: Isolation of fungal strains was carried out in MGA medium as described above. Out of many fungal strains, two potent fungal strains were selected based on the extent of pink zones formed. The two strains were morphologically different and were maintained in PDA medium as strain1 and strain 2.

The L-glutaminase assay was determined by Imada *et al.*, 1973 method [28]. Out of the two strains, the strain showed the highest enzyme production of 628.24 U/ml **Fig. 2**. Strain 1 was selected for further characterization.



STRAIN 1 (GREEN COLOUR)



STRAIN 2 (WHITE COLOUR)

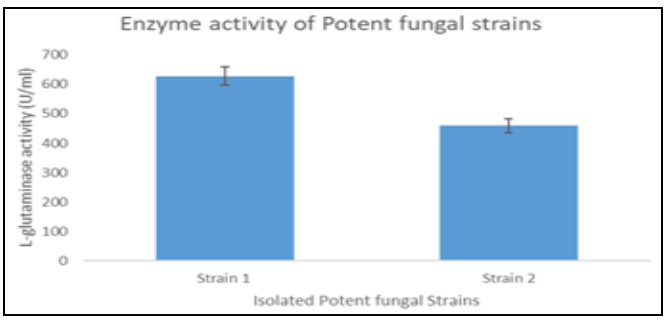


FIG. 2: ENZYME ACTIVITY OF THE TWO STRAINS

Characterization of the Potent Strain 1: The potent strain1 was allowed to grow in PDA medium for candida formation and identified as reported by Diba *et al.*, 2007 [37, 38]. Further, the strain was morphologically characterized by lactophenol cotton blue staining **Fig. 3,** and the morphological analysis of the isolate is summarized in **Table 1**.

TABLE 1: MORPHOLOGICAL CHARACTERISTICS OF STRAIN 1

Sl no.	Morphological Characteristics	Strain1
1	Colour	Green
2	Spores	Thick spores
3	Form	Circular
4	Surface	Rough
5	Margin	White
6	opacity	Transparent
7	Elevation	Convex
8	Reverse view	Green with white lining

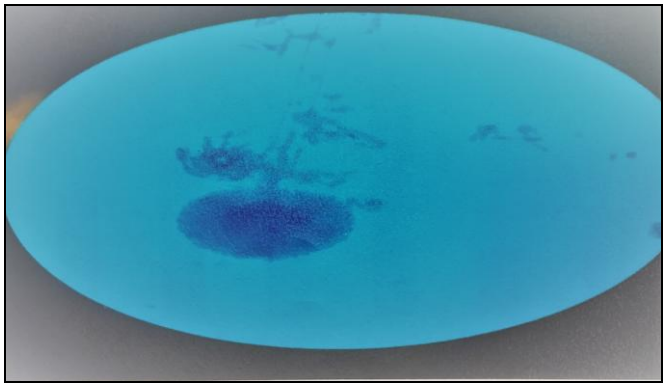


FIG. 3: LACTOPHENOL COTTON BLUE STAINING OF STRAIN1

Based on the morphological characteristics the strain1 belongs to *Aspergillus* species.

The morphological characteristics were in good agreement with the *Aspergillus flavus* strain S4 characteristics described by Hemalatha *et al.* (2018) [38]. The Molecular characterization of strain1 was performed by (ITS)/5.8S rRNA gene sequence analysis, and strain1 was confirmed as *Aspergillus flavus*. The evolutionary history was inferred using the Neighbor-Joining method [30, 50]. The evolutionary distance was computed using the Tamura 3-parameter method [39] and is in the units of the number of base substitutions per site. The rate variation among sites was modeled with a gamma distribution. The analysis involved 12 nucleotides sequences. All positions containing gaps and missing data were eliminated. There was a total of 396 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 [40]. The gene sequence was submitted to the NCBI Gene Bank database with accession number MF445235. The evolutionary relationship of taxa based on ITS sequence is shown in **Fig. 4** and **5.**

GTAGGGTTCCTAGCGAGCCCAACCTCCCACCCGTGTTTACTGT
ACCTTAGTTGCTTCGGCGGGCCCGCCATTCATGGCCGCCGGGG
GCTCTCAGCCCCGGGCCCGCGCCCGCCGGAGACACCACGAACT
CTGTCTGATCTAGTGAAGTCTGAGTTGATTGTATCGCAATCAG
TTAAAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGA
AGAACGCAGCGAAATGCGATAACTAGTGTGAATTGCAGAATTC
CGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTA
TTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCATCAA
GCACGGCTTGTGTGTTGGGTCGTCGTCCCCTCTCCGGGGGGGA
CGGGCCCCAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGC
GTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCGGCGCTT
GCCGAACGCAAATCAATCTTTTCCAGGTTGACCTCGGATCAGG
TAGGGATACCCGCTGAACTTAA

FIG. 4: ITS/5.8S rRNA GENE SEQUENCE DATA OF STRAIN 1

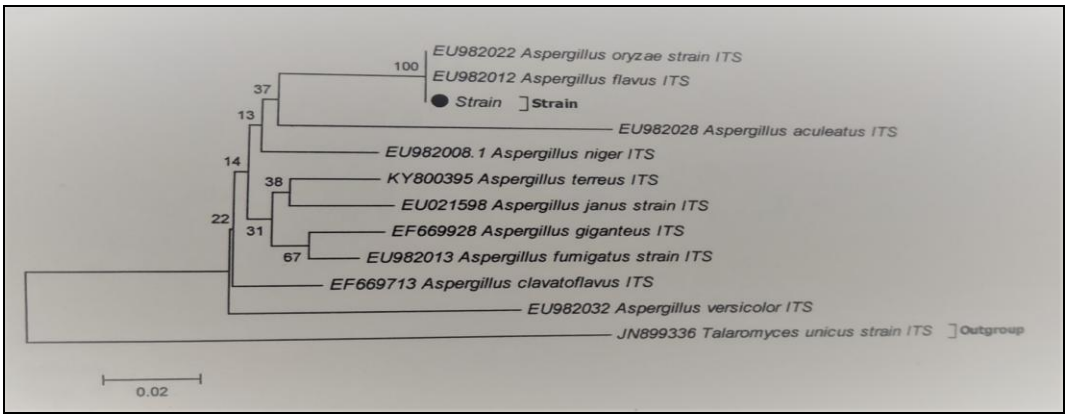


FIG. 5: PHYLOGENETIC EVOLUTIONARY RELATIONSHIP OF TAXA BASED ON ITS SEQUENCE

Selection of Production Media: Among the various production media tested, the Minimal medium showed the maximal L-glutaminase production (628.25 U/ml) by the isolate *Aspergillus flavus* JK-79 **Fig. 6**. The maximal production of L-glutaminase in the Minimal medium may be

attributed to the presence of L-glutamine (inducer) as the sole carbon and nitrogen source in the medium. Other production mediums Czapek Dox, mineral salt glutamine and M9 medium, showed comparatively less L-glutaminase. This may be due to the presence of glucose along with the inducer molecule L-glutamine. Among all the five-production mediums, Mineral salt glutamine showed the least production of L-glutaminase enzyme by marine Aspergillus flavus JK-79. This can be attributed to mineral water added to the medium instead of aged seawater.

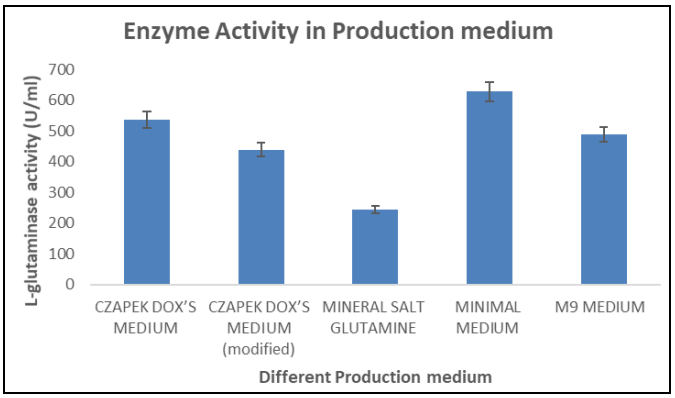


FIG. 6: PRODUCTION OF L-GLUTAMINASE IN DIFFERENT PRODUCTION MEDIUMS BY *ASPERGILLUS FLAVUS* JK-79

Effect of Inoculum size: The Minimal medium was further optimized to enhance the production of L-glutaminase. Various concentrations of inoculum size (1-6%) were analyzed with an incubation period of 144 h at 25℃ **Fig. 7**.

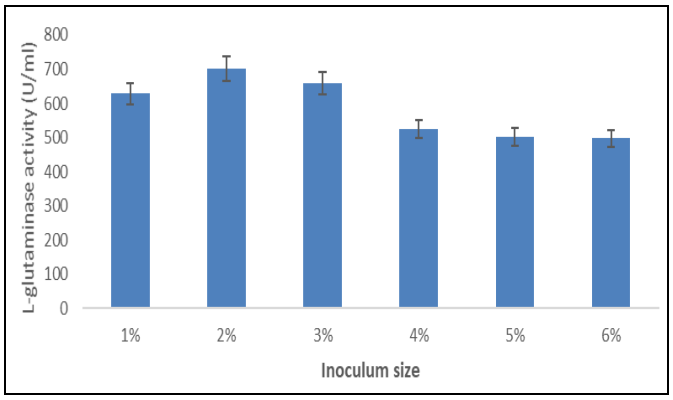


FIG. 7: EFFECT OF INOCULUM SIZE ON L-GLUTAMINASE PRODUCTION BY *ASPERGILLUS FLAVUS* JK-79

Maximal L-glutaminase production was observed at 2% inoculum size (702.03 U/ml). A low concentration of inoculum will not possess sufficient biomass to produce the maximum amount of L-glutaminase enzyme on the other hand very high concentration of inoculum will deplete the medium components and hence will not enhance L-glutaminase production by the isolate *Aspergillus flavus* JK-79 [41]. Sunil Dutt *et al.*, (2013) have reported maximal L-glutaminase production (217.65 IU) by *Aspergillus oryzae* S2 under submerged fermentation at 1% inoculum size [42].

Effect of Temperature: For a varying temperature range (28℃, 30℃, 37℃, 40℃, 45℃ and 50℃), the enzyme production was analyzed and it was found that maximal L-glutaminase production (703 U/mL) was recorded at 25℃ **Fig. 8** and a further increase in temperature decreased the enzyme production. An increase in temperature decreases the L-glutaminase production due to the loss of enzyme activity as temperature increases. A similar result was reported by Sabu *et al.*, (2000)[21] where the maximal L-glutaminase production by marine fungal *Beauveria* sp. was at 27℃. Ahmed *et al.*, (2016) [32] has also reported optimal temperature for the production of L-glutaminase by marine endophytic isolate *Aspergillus sp.* ALAA 2000 as 27℃ under SmF. Nathiya *et al.* (2011) [43] has shown the maximal production of L-glutaminase by terrestrial *Aspergillus flavus KUGF009* at 30℃ by Solid-state fermentation (SSF).

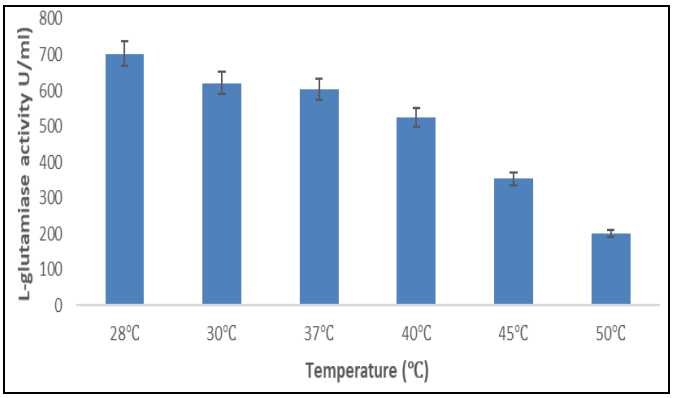


FIG. 8: EFFECT OF TEMPERATURE ON L-GLUTAMINASE PRODUCTION BY *ASPERGILLUS FLAVUS* JK-79

Effect of pH: The effect of pH on L-glutaminase by Aspergillus flavus JK-79 was determined by OFAT and incorporating all other parameters at optimal conditions. It was evident that optimal pH was 8 (790.436 U/ml), and further decrease or increase in pH decreased the L-glutaminase production. From the result **Fig. 9**, it is evident that

the fungal marine isolates Aspergillus flavus JK-79 is alkalophilic in nature as the L-glutaminase production only in the alkaline range. Seawater pH is in the range 7.5 to 8.4; the enzyme production from marine fungal isolate will most probably be maximum in the alkaline range. Sabu *et al.*, (2000) [21] has reported the maximal L-glutaminase production by marine fungal *Beauveria sp*. was at pH 9. Nathiya *et al.*, (2011) [43] and Ahmed *et al.*, (2016)32 have reported maximal L-glutaminase production from terrestrial *Aspergillus flavus* KUGF009 and marine endophytic isolate *Aspergillus sp*. ALAA 2000 respectively at pH 4 in the acidic range. However, Mohamed F. Awad *et al.* (2021) [44] have shown that pH 7 is optimal for L-glutaminase production from *Aspergillus versicolor* Faesay 4.

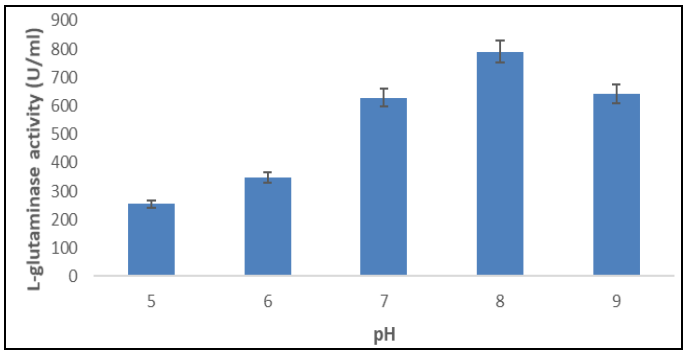


FIG. 9: EFFECT OF PH ON L-GLUTAMINASE PRODUCTION BY *ASPERGILLUS FLAVUS* JK-79

Effect of Additional Carbon, Nitrogen and Amino Acids: The Effect of addition of carbon sources (Glucose, Maltose, Mannitol, Sucrose, Sorbitol, and Mannose), nitrogen sources (Peptone, Yeast extract, Ammonium sulphate, Malt extract, Ammonium nitrate, and Potassium nitrate) and amino acid (L-glutamic acid, L-asparagine, arginine, Methionine, and proline) were analyzed by OFAT methodology.

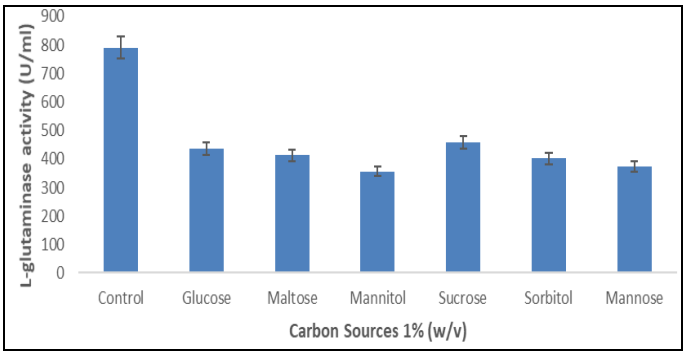


FIG. 10: EFFECT OF ADDITIONAL CARBON SOURCES ON THE PRODUCTION OF L-GLUTAMINASE BY *ASPERGILLUS FLAVUS* JK-79

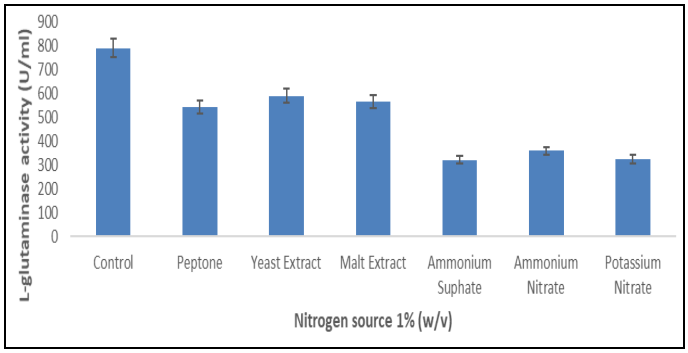


FIG. 11: EFFECT OF ADDITIONAL NITROGEN SOURCES ON THE PRODUCTION OF L-GLUTAMINASE BY *ASPERGILLUS FLAVUS* JK-79

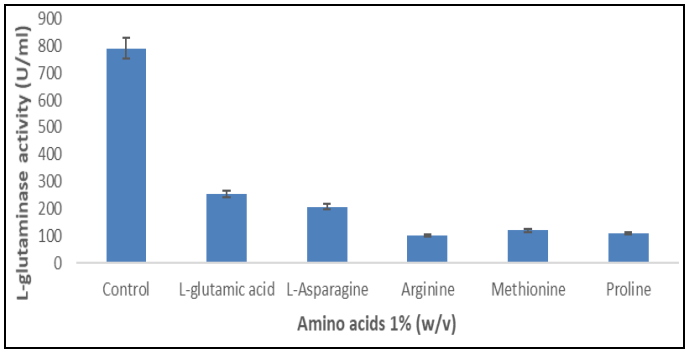


FIG. 12: EFFECT OF AMINO ACIDS ON THE PRODUCTION OF L-GLUTAMINASE BY *ASPERGILLUS FLAVUS* JK-79

From **Fig. 10** and **11,** it is very clear that the addition of carbon and nitrogen sources along with L-glutamine in the concentration of 1% did not enhance the L-glutaminase production by *Aspergillus flavus* JK-79. Since L-glutamine is the sole carbon and nitrogen source and acts as a potent inducer for the enzyme production, further adding extra carbon and nitrogen sources to the production medium did not significantly improve the L-glutaminase production. In **Fig. 12**, the L-glutaminase production has decreased when the L-glutamine is replaced with other amino acids. The results clearly show that in this case, L-glutamine is a potent inducer and a specific substrate for L-glutaminase production.

CONCLUSION: L-glutaminase from marine bacteria has been reported to have a very high affinity for substrate and pH optima in the range 7.4, which is an indication of a better therapeutic regime over the other terrestrial bacterial glutaminase whose pH optima ranges from pH 5-7. Therefore, L-glutaminase produced from marine fungi may hold more potential for treating ALL (Acute Lymphoblastic Leukemia) rather than L-asparaginase from a terrestrial isolate. With this objective, the marine fungal isolate capable of producing L-glutaminase was isolated, and the physiochemical parameters were optimized to obtain the maximal yield of the L-glutaminase enzyme. Thus, maximal production of L-Glutaminase by *Aspergillus flavus* JK-79 was recorded at pH 8 (790.436U/mL), temperature 28°C with 2% inoculum size. Thus, L-glutaminase from *Aspergillus flavus JK-79* could possibly be an alternate for treating ALL with minimal side effects.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

REFERENCES:

1. Sabu A: Sources, Properties and applications of Marine Therapeutic enzymes. Indian J of Biote 2003; 11: 1211-25.
2. Sabu A, Madhavan Nampoothiri K and Pandey A: L-Glutaminase as a Therapeutic Enzyme of Microbial Origin. Microbial Enzymes and Biotrans 2005; 17: 75-90.
3. Neelam Gurung, Sumanta Ray, Sutapa Bose and Vivek Rai: A broader view: microbial enzymes and their relevance in industries, medicine and beyond. Bio Medical Research International 2013; 1-18.
4. Wise DR and Thompson CB: Glutamine addiction: a new therapeutic target in cancer. Trends in Biochemical Science 2010; 35: 427-433.
5. Roberts J, Holcenberg JS and Dolowy WC: Antineoplastic activity of highly purified bacterial glutaminase. Nature 1970; 227: 1136-1137.
6. Bauer K, Bierling R and Kaufman W: Effect of L-glutaminase from *Pseudomonas aureofaciens* in experimental tunors. Naturwissens 1971; 58(10): 526-527.
7. Abell CW and Uren JR: Enzymes In: Anti Tumor Compounds of Natural Origin: Chemistry and Biochemistry. Aszalos A. CRC. Press Boca Raton Florida 1981; 123-153.
8. Raha SK, Roy SK, Dey SK and Chakrabarty SL: Antitumor activity of L-asparaginase from *Cylindrocarpon obtusisporum* MB-10 and its effect on the immune system. Biochemistry International 1990; 21(6): 1001-1011.
9. Pal S and Maity P: Antineoplastic activities of purified bacterial glutaminase on transplanted tumor system. Indian Journal of Cancer Chemotheraphy 1992; 13: 73-76.
10. Dina El-Ghonemy H: Microbial amidases and their industrial applications: A review. Journal of Medical Microbilogy and Diagnosis 2014; 4(173): 1-6.
11. Noura El-Ahmady El-Naggar, Sara El-Ewasy M and Nancy El-Shweihy M: Microbial L-asparginase as a potential therapeutic agent for the treatment of Acute Lymphoblastic Leukemia: The Pros and Cons. International Journal of Pharmacology 2014; 1-18.
12. Swati Sinha and Vinod Kumar Nigam: Production and characterization of L-glutaminase by *Bacillus* sp. International Journal of Pharmaceutical Science and Research 2016; 7(4): 1620-1626.
13. Nandakumar R, Yoshimune K, Wakayama M and Moriguchi M: Microbial Glutaminase: biochemistry, molecular approaches and applications in the food industry. J of Molec Catalysis B: Enzy 2003; 23: 87-100.
14. Krishnakumar S, Alexis Rajan R and Ravikumar S: Extracellular production of L-glutaminase by marine alkalophilic *Streptomyces sp*.-SBU1 isolated from Cape Comorin coast. In J of Geo-Ma Sci 2011; 40(5): 717-721.
15. Unissa R, Sudhakar M, Sunil Kumar Reddy A and Naga Sravanthi K: A review on biochemical and therapeutic aspects of glutaminase. International Journal of Pharmaceutical Science and Research 2014; 5(11): 617-34.
16. Hensley CT, Wasti AT and DeBerardinis RJ: Glutamine and cancer: cell biology, physiology, and clinical opportunities. J of Clinical Investi 2013; 123(9): 3678-84.
17. Sabu A: L-glutaminase production by marine fungi. Ph.D thesis, Cochin University of Science and Technology, Cochin India 1999.
18. Chandrasekaran M: Harnessing of marine microorganisms through soild state fermentation. Journal of Scientific and Industrial Research 1996; 55: 468-471.
19. Moriguchi M, Sakai K, Tateyama R, Faruta Y and Wakayama M: Isolation and characterization of salt tolerant glutaminase from marine *Micrococcus luteus* K3. J of Fermentation and Bioengineering 1994; 77: 621-625.
20. Woo MH, Hak LJ and Storm MC: Hypersensitivity or development of antibodies to asparaginase does not impact treatment outcome of childhood acute lymphoblastic leukemia. J of Clinical Oncology 2000; 18(7): 1525-1532.
21. Sabu A, Keerthi TA, Kumar SR and Chandrasekaran M: L-glutaminase production by marine *Beauveria* sp. under

solid state fermentation. Process Biochemistry 2000; 35: 705-710.
22. Iyer P and Singhal RS: Production of Glutaminase (E.C.3.2.1.5) from *Zygosaccharomyces rouxii*: Statistical optimization using response surface methodology. Bioresource Technology 2008; 99: 4300-4307.
23. Iyer P and Singhal RS: Screening and selection of Marine isolate for L-glutaminase Production and Media Optimization Using response surface Methodology. Applied Biochemistry and Biotechno 2009; 159: 233-250.
24. Iyer P and Singhal RS: Isolation, screening and selection of an L-glutaminase producer from soil and media optimization using a statistical approach. Biotechnology and Bioprocess Engineering 2010; 15: 975-983.
25. Kiruthika J and Saraswathy N: Selective isolation and molecular identification of L-glutaminase producing marine bacteria. Res J of Biotechnology 2013; 8(8): 64-69.
26. Magda M Aly, Roqayah H Kadi, Alia M Aldahlawi, Mayson H Alkhatib and Abdulwahab Noor Wali: Production of the antitumor l-glutaminase enzyme from thermotolerant *Streptomyces* sp. D214, under submerged fermentation conditions. Journal of Experimental Biology and Agricultural Sciences 2017; 5(6): 878-885.
27. Ashraf El-sayed SA: L-glutaminase production by *Trichoderma koningii* under solid state fermentation. Indian Journal of Microbiology 2009; 49: 243-250.
28. Imada A, Igarasi S, Nakahama K and Isono M: Asparginase and Glutaminase activities of microorganisms. J of General Microbiol 1973; 76: 85-99.
29. Kruthi Doriya1 and Devarai Santhosh Kumar: Isolation and screening of L-asparaginase free of glutaminase and urease from fungal sp. 3 Biotech 2016; 6: 239.
30. Saitou N and Nei M: The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 1987; 4: 406-425.
31. Gulati R, Saxena RK, Gupta R: A rapid plate assay for screening of L-asparaginase producing microorganisms. Letters in applied Microbiology 1997; 24: 23-26.
32. Mervat Morsy Abbas Ahmed, Taher M Taha, Nageh F Abo-Dahab and Fareed SM Hassan: Process Optimization of L-Glutaminase Production: a Tumour Inhibitor from Marine Endophytic Isolate *Aspergillus* sp. ALAA-2000. Journal of Microbial and Biochemical Technology 2016; 8(5): 382-389.
33. Renu S, Chandrasekaran M: Extracellular L-glutaminase production by marine bacteria. Biotechnology Letters 1992; 14: 471-74.
34. Kiruthika J and Saraswathy N: Production of L-glutaminase and its optimization from a novel marine isolate *Vibrio azureus* JK-79. African Journal of Biotechnology 2013; 12(50): 6944-6953.
35. Balagurunathan R, Radhakrishnan M and Somasundaram ST: L-glutaminase Producing Actinomycetes from Marine Sediments–Selective Isolation, Semi Quantitative Assay and Characterization of Potential Strain. Australian Journal of Basic and Applied Science 2010; 4: 698-705.
36. Dutta. S, Ghosh S and Pramanik S: L-Asparaginase and L-glutaminase from *Aspergillus fumigatus* WL002: Production and Some Physicochemical Properties, Applied Biochemistry and Microbiology 2015; 51(4): 425–431.
37. Diba K, Kordbacheh P, Mirhendi SH, Rezaie S and Mahmoudi M: Identification of *Aspergillus* species using morphological characteristics. Pakistan Journal of Medical Sciences 2007; 23(6): 867.
38. Hemalatha V, Kalyani P, Chandana Vineela K and Hemalatha K.P.J: Isolation and identification of L-glutaminase producing fungi from agricultural soil. International Journal of Current Advanced Research 2018; 7(12E): 16712-16717.
39. Tamura K: Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+C-content bases. Molecular Biology and Evolution 1992; 9: 678-687.
40. Tamura K, Stecher G, Peterson D, Filipski A and Kumar S: MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 2013; 30: 2725-2729.
41. Kashyap P, Sabu A, Pandey A, Szakacs G and Soccol CR: Extracellular L-glutaminase production by *Zygosaccharomyces rouxii* under solid-state fermentation. Process Biochemistry 2002; 38: 1431-1436.
42. Sunil Dutt PLNSN, Siddalingeshwara KG, Karthic J, Mohsin S Mushtaq, Naveen M Vishwanatha T and Prathiba KS: An approach on microbial biosynthesis of L-glutaminase: a tumour inhibitor. Journal of Applied Pharmaceutical Science 2013; 3 (06): 132-135.
43. Nathiya K, Sooraj Nath S, Angayarkanni J and Palaniswamy: Optimised production of L-glutaminase: A tumour inhibitor from *Aspergillus flavus* cultured on agroindustrial residues. African Journal of Biotechnology 2011; 10(63): 13887-13894.
44. Awad MF, El-Shenawy FS and El-Gendy MMAA: Purification, characterization and anticancer and antioxidant activities of L-glutaminase from *Aspergillus versicolor* Faesay4. Int Microbiol 2021; 24: 169–181. https://doi.org/10.1007/s10123-020-00156-8.
45. Khalil MS, Moubasher MH, El-Zawahry MM and Miche MM: Evaluation of antitumor activity of fungal L-glutaminase produced by Egyptian isolates. Lett Appl Nano Bio Sci 2020; 9(1): 924–930.
46. Masisi BK, El Ansari R, Alfarsi L, Rakha EA, Green AR and Craze ML: The role of glutaminase in cancer. Histopathology 2020; 76(4): 498–508.
47. Vo TD, Sulaiman C, Tafazoli S, Lynch B, Roberts A, Chikamatsu G: Safety assessment of glutaminase from *Aspergillus niger*. Food Sci Nutr 2020; 8: 1433–1450.
48. El-Gendy MMAA, Taher MT, Nageh FA and Fareed HSM: Process optimization of L-glutaminase production; a tumour inhibitor from marine endophytic isolate Aspergillus sp. ALAA-2000. Int J PharmTech Res 2016; 9(8): 256–267.
49. Kumar S, Stecher G, Li M, Knyaz C and Tamura K: MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 2018; 35: 1547–1549.
50. El-Gendy MMAA, Yahya SMM, Hamed AR, Soltan MM and El-Bondkly AMA: Phylogenetic analysis and biological evaluation of marine endophytic fungi derived from Red Sea sponge Hyrtios erectus. Appl Biochem Biotechnol 2018; 185: 755–777.

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