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Production, Optimization and Characterization of L-Glutaminase from Soil-Derived Aspergillus Species under Solid-State Fermentation

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Abstract

L-glutaminase (EC 3.5.1.2) is an amidohydrolase enzyme of significant biomedical and industrial importance due to its potential therapeutic application in cancer treatment and its role in food biotechnology. The present study aimed to isolate, produce, purify, and characterize L-glutaminase from Aspergillus species obtained from soil samples. Fungal isolates were screened using a modified Czapek's medium supplemented with L-glutamine and Congo red dye indicator. Positive isolates were further confirmed by thin layer chromatography for enzymatic activity. Quantitative estimation of enzyme activity was carried out using Nessler's reagent by measuring ammonia released from L-glutamine hydrolysis. Optimization studies were performed to evaluate the influence of physicochemical parameters such as pH, temperature, moisture content, inoculum size, and incubation period on enzyme production. Among the screened isolates, strain S3 showed the highest enzyme activity and was identified as Aspergillus species through microscopic examination. Maximum enzyme activity of 15.4 µmol/ml/min was observed at pH 5.0, 30°C, and 60% moisture content under solid-state fermentation. The enzyme exhibited high specificity toward L-glutamine and demonstrated stability across a range of environmental conditions. The results indicate that Aspergillus species can serve as a promising microbial source for L-glutaminase production with potential applications in oncology, biosensor development, and food fermentation industries.

Keywords: L-glutaminase, anti-tumor agent, microbial enzymes, fermentation, oncology L-Glutaminase: Therapeutic and Industrial Applications.

Introduction

Enzymes are biological catalysts that accelerate biochemical reactions in living organisms. They are essential for metabolic processes and are widely used in industrial, pharmaceutical, and food biotechnology. Microorganisms are considered the most important sources of industrial enzymes because of their rapid growth, ease of cultivation, and ability to produce large quantities of extracellular enzymes[1,2].

Microbial enzymes have gained considerable attention due to their applications in medicine, food processing, and biotechnology. Therapeutic enzymes are used as anticancer agents, thrombolytics, antimicrobials, and digestive aids[2]. Among these, L-glutaminase (EC 3.5.1.2) is an important enzyme that catalyzes the hydrolysis of L-glutamine to L-glutamate and ammonia[3].

L-glutaminase has significant therapeutic importance because it deprives tumor cells of glutamine, an essential nutrient required for their growth and survival[4,5]. Due to this property, the enzyme has been widely investigated for the treatment of lymphosarcoma and acute lymphoblastic leukemia[6].

Microbial sources such as bacteria, fungi, and actinomycetes are widely studied for L-glutaminase production because they provide higher yield and easier downstream processing[7]. Among fungal organisms, *Aspergillus* species have been reported as efficient producers of extracellular enzymes including L-glutaminase[8].

Apart from medical applications, L-glutaminase is also used in the food industry as a flavor-enhancing enzyme, especially in fermented food products such as soy sauce[9]. Therefore, the present study focuses on the isolation, production, purification, and characterization of L-glutaminase from *Aspergillus* species[10].

Materials and Methods

Collection of Soil Samples: Soil samples were collected from diverse ecological niches including Cheemandahalli, Hoskote, Bangalore and the Yeshwanthpura industrial area, Karnataka. Samples were collected in sterile zip-lock pouches and transported to the laboratory to avoid external contamination. Soil is considered one of the most important sources for isolating enzyme-producing microorganisms[3].

Isolation and Screening of Fungi: Fungal isolates were obtained using the serial dilution technique. One gram of soil sample was suspended in 10 ml sterile distilled water and serially diluted. Dilutions of 10^{-3} and 10^{-4} were spread on

Czapek's agar medium and incubated at 28°C for five days. Isolation of enzyme-producing microorganisms using selective media has been widely reported in previous studies[6,7].

Primary Screening (pH Indicator Method): Pure fungal isolates were streaked on modified Czapek's medium containing L-glutamine as the sole carbon and nitrogen source supplemented with Congo red dye indicator. Production of extracellular L-glutaminase resulted in ammonia release causing a pH change in the medium and formation of a pink zone around the colony. Similar screening methods have been previously used for detection of glutaminase producing microorganisms[3,6].

Secondary Screening (Thin Layer Chromatography): Thin Layer Chromatography (TLC) was performed to confirm enzyme production using silica gel as the stationary phase and phenol-water as the solvent system. Amino acids produced during glutamine hydrolysis were detected using ninhydrin reagent[6].

Crude enzyme extract obtained after fermentation was initially analyzed for L-glutaminase activity. In the present study, purification was limited to preliminary confirmation using thin layer chromatography (TLC). Advanced purification techniques such as ammonium sulfate precipitation, dialysis, and ion-exchange chromatography (e.g., DEAE-cellulose) were not performed.

These standard purification procedures are widely employed to achieve higher enzyme purity, remove contaminating proteins, and enable detailed biochemical characterization. Their inclusion in future studies would facilitate determination of enzyme homogeneity and molecular properties.

Identification of Isolates: Morphological identification of fungal isolates was carried out using Lactophenol Cotton Blue staining. Microscopic examination of mycelia, conidia and spore arrangements was performed under a compound microscope to identify the fungal species.

Quantitative Estimation of L-Glutaminase: Enzyme activity was quantified by estimating ammonia released from L-glutamine using Nessler's reagent. Similar colorimetric methods have been widely used for determining glutaminase activity in microbial cultures[11].

One unit of enzyme activity was defined as the amount of enzyme required to release one micromole of ammonia per minute under standard assay conditions.

Results and Discussion

Screening of L-Glutaminase Producing Fungi: Five fungal isolates were screened for L-glutaminase production using Congo red dye indicator. Among the isolates, three cultures showed positive results indicated by the formation of a pink halo around the colonies. Similar screening results for L-glutaminase producing fungi have been reported previously[3,12].

Identification of Fungal Isolates: Microscopic observation of isolate S3 using Lactophenol Cotton Blue staining revealed characteristic morphological features of *Aspergillus* species. Previous studies have also reported *Aspergillus* species as efficient producers of extracellular L-glutaminase[3].

Optimization of Culture Conditions: Moisture Content: Maximum enzyme activity (15.4 µmol/ml/min) was observed at 60% moisture content. Enzyme production varied with moisture levels ranging from 40–65%. Similar moisture optimization studies have been reported for microbial enzyme production under solid state fermentation[13].

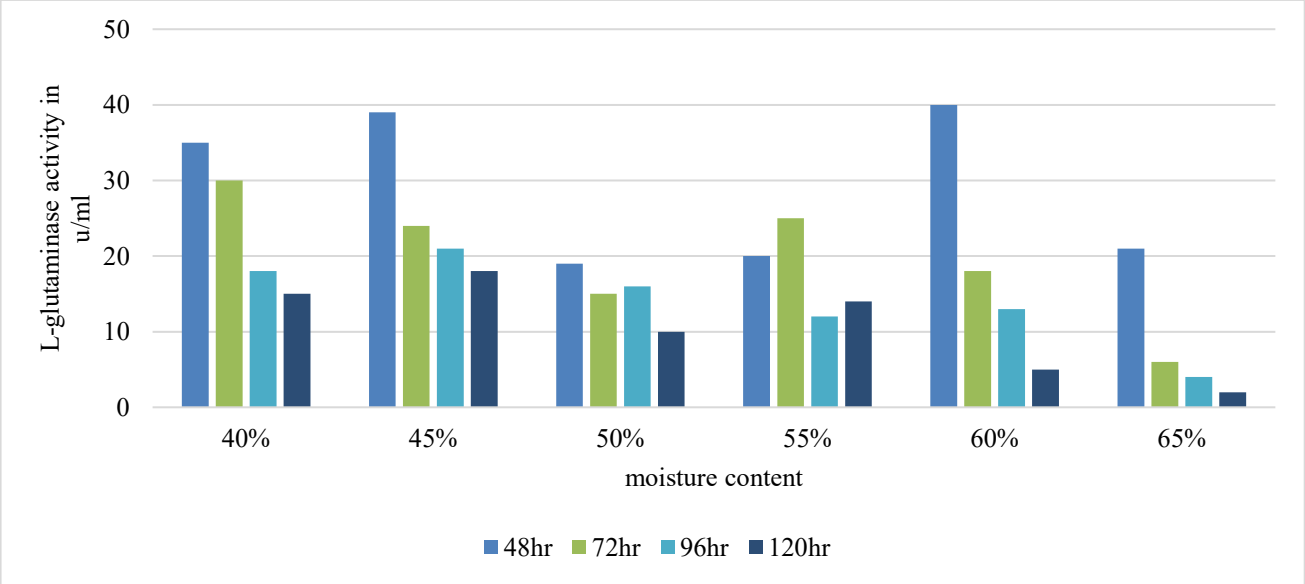

Figure-1: L-Glutaminase activity observed at different Moisture content.

pH: The results indicated that pH 5.0 was optimal for enzyme production. Previous studies on *Pseudomonas fluorescens* and *Aspergillus oryzae* also reported acidic pH conditions favorable for L-glutaminase production[4,12].

Temperature: The optimal initial temperature for L-glutaminase production was found to be 30°C, at which maximum enzyme activity of 11.88 µmol/ml/min was observed. Enzyme activity decreased gradually with further increase in incubation temperature. Similar optimal temperature ranges have been reported for microbial glutaminase enzymes[14].

Optimization of Inoculum Size: The optimum inoculum concentration was observed to produce significant enzyme activity. Previous studies have shown that inoculum size significantly affects microbial enzyme production in fermentation systems[4]. The optimal initial inoculum size for L-glutaminase production was determined based on enzyme activity, with a maximum activity of 11.24 µmol/ml/min observed. Similar findings have been reported for *Aspergillus flavus*, where enzyme production varied with inoculum concentrations ranging from 1.0 to 3.0.

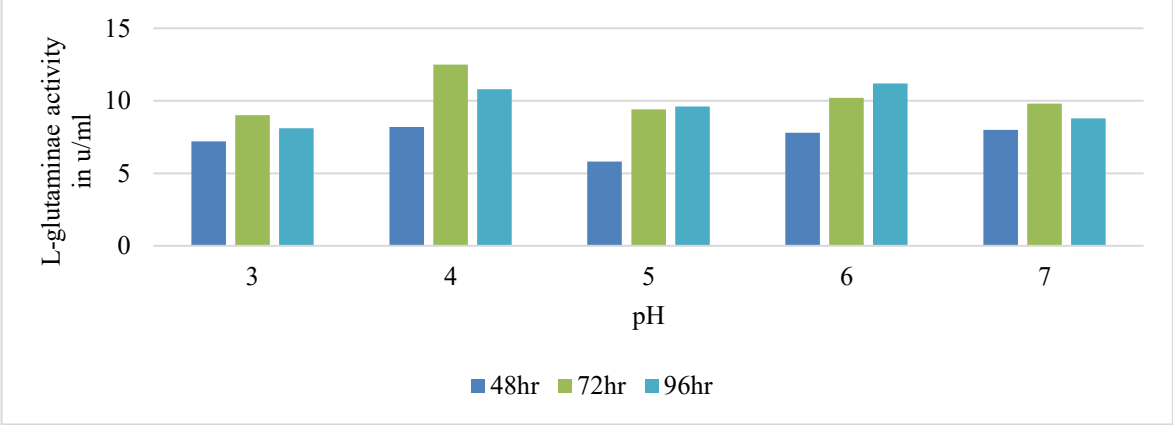

Figure-2: L-Glutaminase activity observed at different pH.

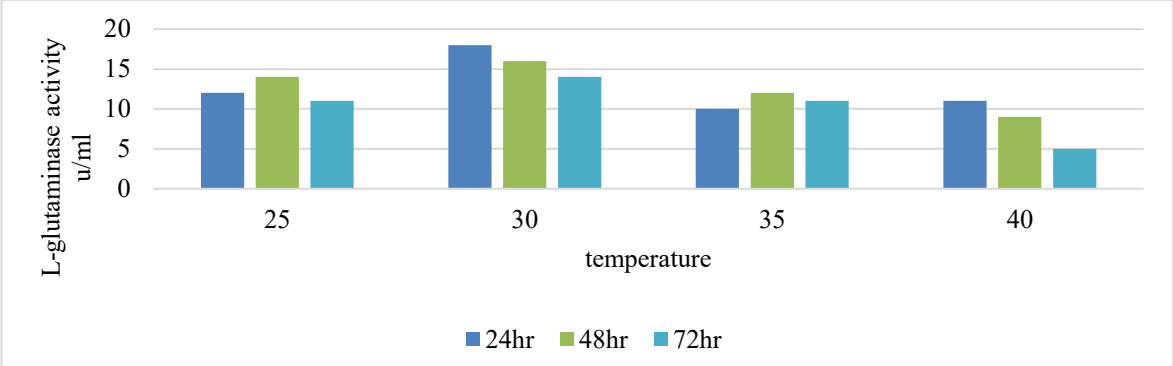

Figure-3: L-Glutaminase activity observed at different temperature.

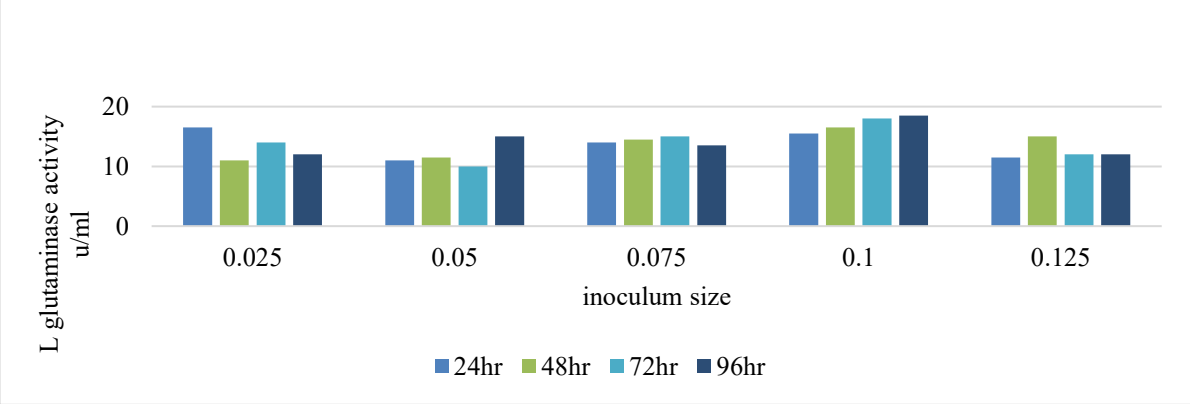

Figure-4: L-Glutaminase activity observed at Inoculum size.

Thin Layer Chromatography: Thin layer chromatography confirmed the presence of L-glutaminase by detecting hydrolysis products of glutamine. Similar chromatographic confirmation methods have been used in earlier studies[6].

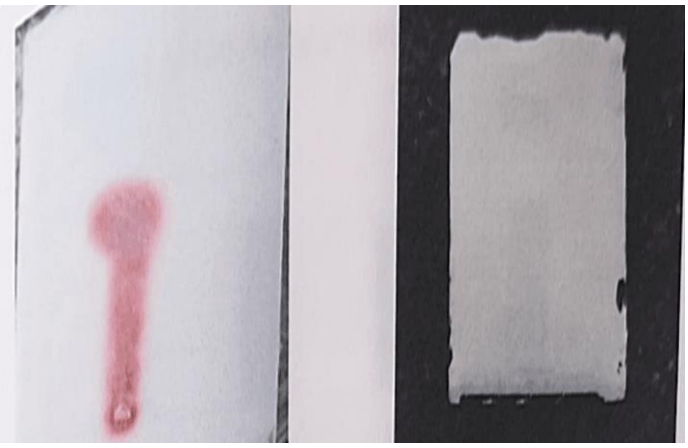

Figure-4: Thin Layer chromatography plate showing presence of L-Glutaminase.

Enzyme activity was confirmed by the hydrolysis of glutamine, indicating L-glutaminase production by *Aspergillus* sp.

The enzyme preparation used in this study represents a crude extract. Although TLC confirmed enzymatic activity through detection of glutamine hydrolysis products, the absence of advanced purification steps limits detailed characterization of the enzyme.

Discussion: The present study demonstrated that fungal isolates obtained from soil samples are capable of producing extracellular L-glutaminase under suitable fermentation conditions. Soil environments are known to harbor diverse microbial communities capable of synthesizing various industrially important enzymes. Several studies have reported that microorganisms isolated from soil, particularly fungi and actinomycetes, are efficient producers of L-glutaminase due to their metabolic versatility and ability to secrete extracellular enzymes in significant quantities[1,3].

Among the screened isolates, the fungal strain designated as S3 exhibited comparatively higher enzyme activity. Microscopic characterization suggested that the isolate belonged to the genus *Aspergillus*. Species of *Aspergillus* are widely recognized for their ability to produce industrial enzymes and secondary metabolites, and they have been extensively used in fermentation-based enzyme production systems[15,16]. Previous reports have also highlighted the potential of *Aspergillus* species as promising microbial sources for L-glutaminase production with applications in pharmaceutical and food industries[6].

Optimization of fermentation parameters revealed that enzyme production was significantly influenced by environmental conditions such as moisture content, pH, temperature, and inoculum size. In the present investigation, maximum enzyme activity was observed at 60% moisture content. Moisture plays a crucial role in solid-state fermentation systems by regulating nutrient diffusion, oxygen transfer, and microbial metabolic activity. Similar findings have been reported in earlier studies where optimal moisture levels significantly enhanced enzyme production during solid-state fermentation processes[15,16].

The results also indicated that pH 5.0 was optimal for L-glutaminase production by the fungal isolate. The acidic pH requirement observed in this study is consistent with previous reports describing optimal enzyme production by fungal species under slightly acidic conditions[11]. The pH of the medium influences enzyme stability, substrate availability, and transport of nutrients across the microbial cell membrane, thereby affecting microbial growth and metabolite synthesis.

Temperature optimization experiments showed that maximum enzyme activity occurred at approximately 30°C. Temperature is an important factor affecting microbial metabolism and enzymatic reactions. Many fungal enzymes exhibit optimal activity within the temperature range of 25–35°C, which corresponds with the observations of the present study[8]. At higher temperatures, enzyme activity may decrease due to denaturation of proteins or inhibition of microbial growth.

Thin layer chromatography analysis confirmed the hydrolysis of L-glutamine into L-glutamate, thereby indicating the presence of active L-glutaminase enzyme in the culture extract. Chromatographic techniques are widely used for qualitative confirmation of enzymatic reactions and substrate conversion in biochemical studies[17].

Another significant observation in the present study is the substrate specificity of the purified enzyme. The enzyme showed strong specificity toward L-glutamine compared with other tested substrates. Substrate specificity is an important characteristic that determines the suitability of enzymes for industrial and pharmaceutical applications. Microbial L-glutaminase enzymes have been reported to possess high specificity toward L-glutamine, which contributes to their therapeutic effectiveness in cancer treatment[7,18].

The therapeutic potential of L-glutaminase is primarily associated with its ability to deprive tumor cells of glutamine, an essential nutrient required for their rapid proliferation. Tumor cells rely heavily on extracellular glutamine for energy metabolism and biosynthesis of nucleotides and proteins. By hydrolyzing glutamine into glutamate and ammonia, L-glutaminase disrupts tumor cell metabolism and inhibits cancer cell growth[5]. Recent studies have further emphasized the role of microbial L-glutaminase as a promising anticancer enzyme for the treatment of leukemia and other glutamine-dependent tumors[7,19]. In addition to medical applications, L-glutaminase also plays an important role in the food industry. The enzyme is widely used as a flavor-enhancing agent in fermented food

products such as soy sauce and other protein-rich foods[9]. The conversion of glutamine to glutamic acid contributes to the development of desirable umami flavor in fermented foods.

Overall, the findings of the present study suggest that *Aspergillus* species represent a promising microbial source for L-glutaminase production using solid-state fermentation techniques. Optimization of fermentation parameters significantly enhanced enzyme yield, highlighting the importance of environmental factors in microbial enzyme production.

A limitation of the present study is the lack of extensive purification of L-glutaminase. While thin layer chromatography confirmed enzyme activity, it does not provide sufficient evidence of enzyme purity or molecular characteristics. Standard purification methods such as ammonium sulfate precipitation, dialysis, and ion-exchange chromatography (DEAE-cellulose) are essential for achieving purified enzyme fractions.

Further purification using chromatographic techniques is required to determine enzyme homogeneity, molecular weight, and kinetic parameters. Incorporation of these methods in future studies will enhance the reliability of biochemical characterization and support potential industrial and therapeutic applications.

Conclusion

This study confirms that Aspergillus species are a promising source to produce L-glutaminase. Optimal conditions for maximum enzyme production were identified as pH 5.0, temperature 25°C, and appropriate moisture content. The enzyme showed high stability, specificity, and activity, making it suitable for therapeutic and industrial applications. The use of solid-state fermentation with low-cost substrates enhances production efficiency. Overall, L-glutaminase has strong potential as an anti-cancer agent and in industrial processes. However, further purification and kinetic characterization are required to validate its suitability for large-scale and clinical applications.

References

1. Abdallah, A. N., Amer, K. S., & Habeeb, K. M. (2013). Production, purification and characterization of L-glutaminase enzyme from *Streptomyces avermitilis*. *African Journal of Microbiology Research,* 7(14), 1184–1190.

2. Anita, S., Namita, S., &Narsi, R. B. (2009). Production of cellulases by *Aspergillus heteromorphus* from wheat straw under submerged fermentation. *International Journal of Civil and Environmental Engineering,* 1(1), 23–26.

3. Balagurunathan, R., Radhakrishnan, M., & Somasundaram, S. T. (2010). L-glutaminase producing actinomycetes from marine sediments: Selective isolation, semi-quantitative assay and characterization of potential strain. *Australian Journal of Basic and Applied Sciences,* 4, 698–705.

4. Chitanand, M. P., & Shete, H. G. (2012). Condition optimization and production of extracellular L-glutaminase from *Pseudomonas fluorescens*. *International Journal of Pharmacy and Biological Sciences,* 3(3), 155–162.

5. Divya Teja, D., Sri Devi, V., Harsha, N., Satya Vishala, S., & Santhosha Lakshmi, P. K. (2014). Production of L-glutaminase from marine ecosystems and optimal conditions for maximal production by actinomycetes. *International Journal of Advanced Research,* 2(1), 485–490.

6. Cooper, G. M. (2000). *The cell: A molecular approach* (2nd ed.). ASM Press.

7. Garcia-Kirchner, O., Muñoz-Aguilar, M., Pérez-Villalva, M., & Huitrón-Vargas, C. (2002). Mixed submerged fermentation with two filamentous fungi for cellulolytic and xylanolytic enzyme production. *Applied Biochemistry and Biotechnology,* 98(1–9), 1105–1114.

8. Lonsane, B. K., Ghildyal, N. P., Budiatman, S., & Ramakrishna, S. V. (1985). Engineering aspects of solid-state fermentation. *Enzyme and Microbial Technology, 7*(6), 258–265.

9. Huerta-Saquero, A., Calderón, J., Arreguín, R., Calderón-Flores, A., & Durán, S. (2001). Over expression and purification of *Rhizobium etli* glutaminase A by recombinant and conventional procedures: A comparative study of enzymatic properties. *Protein Expression and Purification,* 21(3), 432–437.

10. Iyer, P., & Singhal, R. (2010). Isolation, screening, and selection of an L-glutaminase producer from soil and media optimization using a statistical approach. *Biotechnology and Bioprocess Engineering,* 15, 975–983.

11. Iyer, P., & Singhal, R. S. (2009). Screening and selection of marine isolate for L-glutaminase production and media optimization using response surface methodology. *Applied Biochemistry and Biotechnology,* 159(1), 233

12. Jeon, J. M., Lee, H. L., & Han, S. H. (2009). Partial purification and characterization of glutaminase from *Lactobacillus reuteri* KCTC3594. *Applied Biochemistry and Biotechnology*.

13. Kashyap, P., Sabu, A., Pandey, A., Szakacs, G., &Soccol, C. R. (2002). Extracellular L-glutaminase production by *Zygosaccharomyces rouxii* under solid-state fermentation. *Process Biochemistry,* 38(3), 307–312.

14. Bobbarala, V., Prabhakar, T. P., & Guntuku, G. S. (2009). Screening of L-glutaminase-producing marine bacterial cultures for extracellular production of L-glutaminase. *International Journal of Chemical Technology Research*.

15. Kozolov, T. A., Tsvetkova, T., et al. (1982). Photosensitized oxidation of asparagine-glutamine deamidase from *Pseudomonas fluorescens*. *Bulletin of Experimental Biology and Medicine,* 94(3), 1209–1212.

16. Kusaykin, M. I., et al. (2003). Distribution of O-glycosylhydrolases in marine invertebrates: Enzymes of the marine mollusk *Littorina kurila* that catalyze fucoidan transformation. *Biochemistry (Moscow),* 68(3), 317–324.

17. Papaspyridi, L. M., Aligiannis, N., Topakas, E., Christakopoulos, P., Skaltsounis, A. L., & Fokialakis, N. (2012). Submerged fermentation of the edible mushroom *Pleurotusostreatus* in a batch stirred tank bioreactor as a promising alternative for the effective production of bioactive metabolites. *Molecules,* 17(3), 2714–2724.

18. Levine, A. J., & Puzio-Kuter, A. M. (2010). The control of the metabolic switch in cancers by oncogenes and tumor suppressor genes. *Science,* 330(6009), 1340–1344.

19. Liu, S. L., Shi, D. Y., Shen, Z. H., & Wu, Y.D. (2000). Effects of glutamine on tumor growth and apoptosis of hepatoma cells. *Acta Pharmacologica Sinica,* 21, 668–672.

20. Lokendrakumar, Balvinder, S., Dilipkumar, A., Joydeep, M., & Debashish, G. (2012). A temperature- and salt-tolerant L-glutaminase from the Gangotri region of Uttarakhand Himalaya: Enzyme purification and characterization. *Applied Biochemistry and Biotechnology,* 166, 1723–1735.

21. Prabhakar, A., Krishnaiah, K., Janaun, J., & Bono, A. (2005). An overview of engineering aspects of solid-state fermentation. *Malaysian Journal of Microbiology,* 1(2), 10–16.

22. Sivakumar, K., Sahu, M. K., Manivel, P. R., & Kannan, L. (2006). Optimum conditions for L-glutaminase production by an actinomycete strain isolated from the estuarine fish *Chanoschanos* (Forsskål, 1775). *Indian Journal of Experimental Biology,* 44(3), 256–260.

23. Wakayama, M., Yamagata, T., Kamemura, A., Bootim, N., Yano, S., Tachiki, T., Yoshimune, K., & Moriguchi, M. (2005). Characterization of salt-tolerant glutaminase from *Stenotrophomonas maltophilia* NYW-81 and its application in Japanese soy sauce fermentation. *Journal of Industrial Microbiology & Biotechnology,* 32(9), 383–389.