Research Article

Solid-state fermentation of corn husk for the synthesis of Asparaginase by *Fusarium oxysporum*

Chanakya Pallem*

Department of Microbiology, Ganesh Scientific Research Foundation (GSRF), Kirti Nagar, New Delhi-110015, India

Received: 19 November 2018 Revised: 10 December 2018 Accepted: 2 January 2019

Abstract

Background: Agro-wastes are potential alternative raw materials for the production of several value-added metabolites. Solid-state fermentation is used for microbial production of bioactive compounds especially enzymes, due to its advantages, such as usage of low-cost raw materials, less effluent generation, and production of biocatalysts with unique catalytic properties. **Objective:** This study mainly focuses on the optimization of process parameters for the synthesis of asparaginase in solid-state fermentation by *Fusarium oxysporum* (NCIM 1008) in a production medium, containing corn husk as substrate. **Methods:** Production of asparaginase in solid-state fermentation by *Fusarium oxysporum* (NCIM 1008) was investigated. The impact of initial moisture content, fermentation time, initial pH, and fermentation temperature, effect of additional carbon and nitrogen sources, on the production of enzyme was studied. **Results:** Maximal enzyme activity (27.10 U/gds) with corn husk was achieved with following optimised conditions: initial moisture content 65% (v/w), initial pH 6.0, supplemented with xylose 0.5% (w/w), urea 1% (w/w) incubated at 28°C for 72 h. **Conclusion:** Even though corn husk is high in cellulose content, it supported better microbial cell growth with good enzyme productivity. After optimization, asparaginase activity obtained is comparable with the activity of asparaginases, synthesised from other fungal strains. The process optimization and inexpensive substrate usage makes solid-state fermentation more economical for the production of asparaginase.

***Keywords*:** Asparaginase, *Fusarium oxysporum*, Solid-state fermentation, Corn husk

Introduction

Over the past several decades, usage of agricultural by-products has gained more significance in various bioprocesses due to their rich nutrient content and low cost. These agro-wastes generally include molasses, oil cakes, husk, straw, peels of fruits and vegetables, bagasse and other agro-materials generated during their processing have been reported for the production of many value-added products. Transforming these nutritionally rich by-products into useful bio-products by fermentation technologies not only minimizes the production cost of the process but also lowers the risk of environmental pollution (Pandey et al., 1999).

L-asparagine amidohydrolase (EC 3.5.1.1), most commonly known as asparaginase is an enzyme of wide spectrum therapeutic applications. It is potentially used in the treatment of various forms of leukaemia (Anjana et al., 2018; Umesh et al., 2007). Asparaginase is also used in food industry to prevent the acryl amide formation when foods are processed in high temperatures (Cachumba et al., 2016). Traditionally, submerged fermentation (SmF) process has been widely used for the production of asparaginase. To overcome the drawbacks of SmF, solid-state fermentation (SSF) has come into existence as an alternate economical process for the synthesis of various bioproducts by utilizing the agro-waste materials (Pandey et al., 1999; Sadh et al., 2018). SSF has been employed for production of various microbial metabolites (Rojan et al., 2006; Sandhya et al., 2005; Corona et al., 2005; Pandey et al., 1999; Sarada and Sridhar, 1998). As per the documented literature, several species microbial genera (Kruthi and Kumar, 2018; Ghosh et al., 2013; Baskar and Renganathan, 2011; Seyedeh et al., 2011; Kumar et al., 2010; Mishra, 2006; Saleem Basha et al., 2009; Abdel-Fattah and Olama, 2002; Kil et al., 1995) have been reported for the production of asparaginase.

Corn husk is a thin cellulose-rich leafy sheath that covers the

Address for Corresponding Author:
Chanakya Pallem
Department of Microbiology,
Ganesh Scientific Research Foundation (GSRF), Kirti Nagar, New Delhi-110015, India
E-mail ID: pallemchanakya@yahoo.com

DOI: https://doi.org/10.31024/ajpp.2019.5.4.5

corn cobs. It contains high cellulose content and was tried to exploit for different applications such as a potential source of anthocyanins (Li et al., 2008), as substrate for citric acid (Hang and Woodams, 2000) and rifamycin B (Mahalaxmi et al., 2010) production. Even though it has been associated with so many applications, still it is common practice to dispose-off corn husk along with corn stalk and leaves either by burning or tilling in to the soil in India. Owing to its abundant availability, renewable nature, easy release of carbon sources and high water holding capacity, the role of corn husk as the solid substrate for asparaginase production by *Fusarium oxysporum* (NCIM 1008) was investigated in the present study.

Materials and Methods

Materials

Corn husk was collected from the nearby agricultural fields of National Capital Region (NCR), India. Before use, it was washed thoroughly and dried in an oven at 60-80°C for one day. The dried substrate was ground and made in to particle size of 1mm. All the chemicals used in this research work were of analytical grade and purchased from Sigma-Aldrich, Bangalore and nutrient media from Hi-media Laboratories, Mumbai.

Microorganism

Fusarium oxysporum (NCIM 1008) received from NCIM, Pune was used. The culture was maintained on Potato Dextrose agar (PDA) medium slants at 28°C for one week. The slants were stored at 4°C and were sub-cultured monthly. Under aseptic conditions, fungal conidial suspension was prepared from a freshly raised seven day old culture of *F. oxysporum* by suspending in 10 ml of 0.85% sterile saline solution. This is used as inoculum for subsequent fermentation experiments.

Solid-state fermentation of corn husk

Corn husk (5g) was taken into 250 ml of Erlenmeyer flasks, moistened with 3ml of the mineral salt solution (composition in g/L: KH_2PO_4 0.1, NaCl 0.25, $MgSO_4$ 0.01, $CaCl_2$ 0.01) and autoclaved. After cooling to room temperature, the flasks were inoculated with 2 ml of the fungal conidial suspension aseptically. The contents in the flasks were uniformly mixed and incubated in an incubator at 28°C for about one week (fermentation time) respectively.

Enzyme extraction and assay

Crude enzyme was extracted by using 0.1M phosphate buffer (Ghosh et al., 2013). The activity of L-asparaginase was estimated by measuring the amount of ammonia released by nesslerization method (Wriston and Yellin, 1973). One Unit (U) of L-asparaginase is defined as the amount of enzyme required to liberate one µmole of ammonia under optimal assay conditions.

Optimization studies of SSF

SSF was optimized using single-parameter optimization technique. Various crucial process parameters such as fermentation time (12-168h), initial moisture content (40-80% v/w), initial pH (4.0-10.0, adjusted with 1N HCl or NaOH), incubation temperature (24-36°C) and supplementation of nutritional (both carbon and nitrogen) sources were optimised. Samples were drawn at regular time intervals of 12h and the enzyme assay was carried out to estimate the enzyme activity. All the experiments and assays were run in triplicate and the mean values are noted for better results.

Results and discussion

The selection of a suitable substrate for solid-state fermentation is an important factor because it determines the production cost of the entire process. In this work, corn husk has been selected for the production of asparaginase based on its chemical and nutritional composition, cost and availability.

Optimization of fermentation time

Corn husk (5g), inoculated with 2ml of fungal conidial suspension and the fermentation was run with initial moisture content of 60% (v/w) and incubated at 28°C. Samples were analysed for every 12h and the enzyme activity was reported. The enzyme productivity has shown growth relatedness with incubation time and the maximum enzyme activity (8.22 U/gds) was observed after 72h (Figure 1). Microbial growth and enzyme synthesis were inter- dependent with respect to fermentation time. After 72h, the enzyme activity started to decrease gradually. This reduction might be due to the fact that the microbial strain have reached a stage, from which it could no longer balance its steady growth with the available nutrient resources.

Optimization of initial moisture content

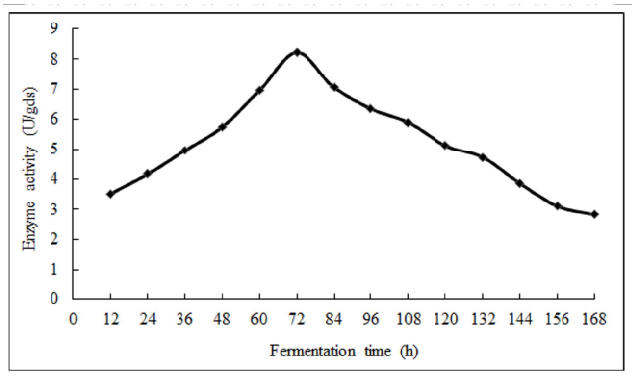

Figure 1. Effect of fermentation time on asparaginase biosynthesis

In SSF, initial moisture content is very important parameter which directly impacts the microbial growth, maximum substrate utilization and enzyme productivity. Maximum enzyme yield (11.31 U/gds) was noticed at 65% v/w (Figure 2) after 72h of fermentation. Decrease in enzyme activity was noticed on either sides of the optimum moisture content. This decrement in enzyme activity might be due to decrease in substrate porosity and presence of contamination at high moisture level (Lonsane et al., 1985).

Optimization of initial pH

Figure 3, shows that maximum asparaginase productivity (16.28 U/gds) at pH 6.0. As per the data, generally agro-wastes possess excellent buffering capacity and that their use offers advantage for SSF processes (Lonsane et al., 1985).Medium pH strongly affects the microbial growth and activity of the metabolites they synthesize. Fungal strains are noted for their best performance in the pH range of 4-7 and lower pH avoids contamination by other microbes such as bacteria.

Optimization of fermentation temperature

SSF was run at different fermentation temperatures varying from 24-36°C. After 72h, the samples were analysed and examined for enzyme activity. The fungal strain has shown better growth and

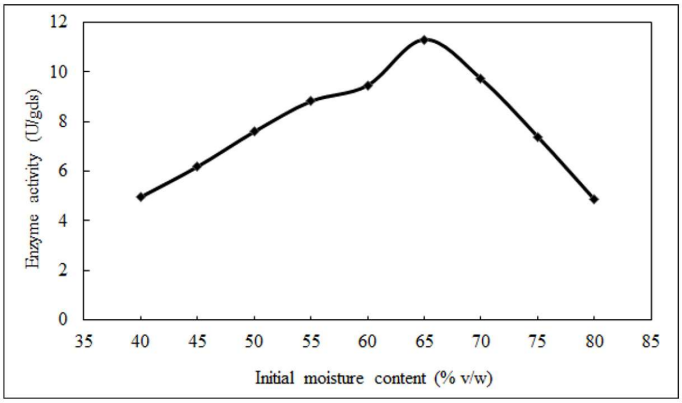

Figure 2. Effect of initial moisture content on asparaginase biosynthesis

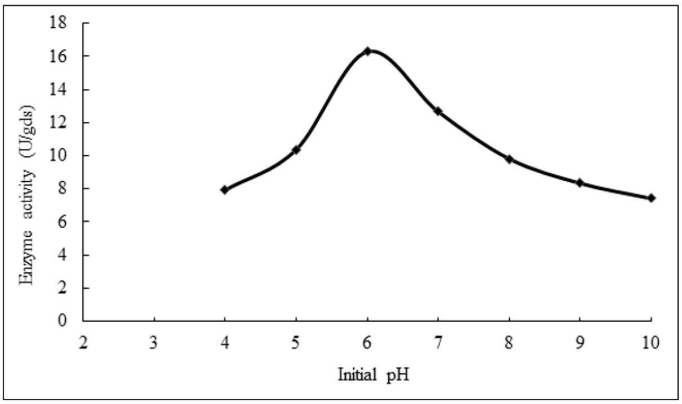

Figure 3. Effect of Initial pH on asparaginase biosynthesis

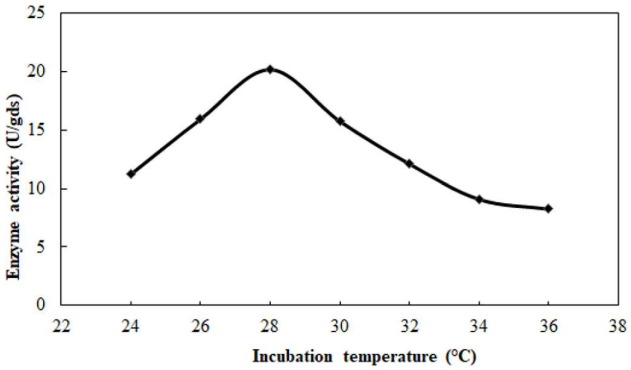

Figure 4. Effect for incubation temperature on asparaginase synthesis

enzyme productivity at 28°C and it was 20.16 U/gds (Figure 4). Temperature is very much useful in determining the effects of protein denaturation, promotion or suppression of a particular metabolite, cell viability and death.

Effect of additional nutritional sources

Among the various available nutritional (both carbon and nitrogen) sources incorporated in the production medium at 1% (w/w), of which xylose as carbon source and urea as nitrogen source has shown some impact on enzyme activity and microbial growth. Attempts were made to estimate the optimum concentration of xylose and urea for maximum enzyme productivity by the fungal culture. The maximal enzyme activity (23.38 U/gds) with 0.5% xylose; 27.10 U/gds with 1% urea was reported (data not shown).

With all the optimized parameters, SSF was run in duplicate and the enzyme activity was analysed. The obtained enzyme activity was 27.1±0.05 U/gds.

Conclusion

The findings of this study suggest that the agro-wastes such as corn husk can be productively utilised as a promising substrate for the production of asparaginase in solid-state fermentation by the fungal strain, *Fusarium oxysporum* NCIM 1008. Furthermore, enzyme activity can be enhanced by developing various scale-up and statistical optimization strategies for its commercial production.

Acknowledgements

The author is thankful to Director, GSRF, New Delhi for supporting the research.

Conflict of Interest

No conflict of interest with regard to this manuscript with anyone in any form.

References

Abdel-Fattah YR, Olama ZA. 2002. L-asparaginase

production by *Pseudomonas aeruginosa* in solid-state culture: Evaluation and optimization of culture conditions using factorial designs. Process Biochemistry, 38:115-122.

Anjana KV, Bhumi S, Dushyant D, Haresh ZP, Haren G, Rakesh R, Bharti PD. 2018. Characterization of L-asparaginase from marine-derived *Aspergillus niger* AKV-MKBU, its anti-proliferative activity and bench scale production using industrial waste. International Journal of Biological Macromolecules, 108:41-46.

Baskar G, Renganathan S. 2011. Design of experiments and artificial neural network linked genetic algorithm for modeling and optimization of L-asparaginase production by *Aspergillus terreus* MTCC 1782. Biotechnology and Bioprocess Engineering, 16:50-58.

Cachumba JJ, Antunes FA, Peres GF, Brumano LP, Santos JC, Da Silva SS. 2016.Current applications and different approaches for microbial l-asparaginase production. Brazilian Journal of Microbiology, 47(1):77-85.

Corona A, Sa'ez D, Agosin E. 2005. Effect of water activity on gibberellic acid production by *Gibberella fujikuroi* under solid-state fermentation conditions. Process Biochemistry, 40:2655-2658.

Ghosh S, Murthy S, Govindasamy S, Chandrasekaram M. 2013. Optimization of L-asparaginase production by *Serratia marcescens* (NCIM 2919) under solid state fermentation using coconut oil cake. Sustainable and Chemical Processes, 1(9):1-8.

Hang YD, Woodams EE. 2000. Corn husks: a potential substrate for production of citric acid by *Aspergillusniger*. LWT-Food Science and Technology, 33:520–1.

Kil JO, Kim GN, Park I. 1995. Extraction of extracellular L-asparaginase from *Candida utilis*. Bioscience Biotechnology and Biochemistry, 59:749-750.

Kruthi D, Kumar SD. 2018. Solid state fermentation of mixed substrate for l-asparaginase production using tray and in-house designed rotary bioreactor. Biochemical Engineering Journal, 138(15):188-196.

Kumar S, Dasu VV, Pakshirajan K. 2010. Localization and production of novel L-asparaginase from *Pectobacterium carotovorum* MTCC 1428. Process Biochemistry, 45:223-229.

Li CY, Kim HW, Won SR, Min HK, Park KJ, Park JY, Ahn MS, Rhee HI. 2008. Corn Husk as a Potential Source of Anthocyanins. Journal of Agricultural and Food Chemistry, 56(23): 11413-11416

Lonsane BK, Ghildyal NP, Budiatman S, Ramakrishna SV. 1985. Engineering aspects of solid-state fermentation. Enzyme Microbial Technology, 7:258-265.

Mahalaxmi Y, Sathish T, SubbaRao Ch, Prakasham RS. 2010. Corn husk as a novel substrate for the production of rifamycin B by isolated *Amycolatopsis* sp. RSP 3 under SSF. Process Biochemistry, 45:47-53.

Mishra A. 2006. Production of L-asparaginase, an anticancer agent, from *Aspergillus niger* using agricultural waste in solid state fermentation. Applied Biochemistry and Biotechnology, 135:3-42.

Pandey A, Selvakumar P, Soccol CR, Nigam P. 1999. Solid-state fermentation for the production of industrial enzymes. Current Science, 77:149-162.

Rojan P, John K, Nampoothiri M, Pandey A. 2006. Solid-state fermentation for L-lactic acid production from agro wastes using *Lactobacillus delbrueckii*. Process Biochemistry, 41:759-763.

Sadh PK, Duhan S, Duhan JS. 2018. Agro-industrial wastes and their utilization using solid state fermentation: a review. Bioresources and Bioprocessing, 5(1):1-15.

SaleemBasha N, Rekha R, Komala M, Ruby S. 2009. Production of extracellular anti-leukaemic enzyme L-asparaginase from marine Actinomycetes by solid state and submerged fermentation: Purification and Characterization. Tropical Journal of Pharmaceutical Research, 8:353-360.

Sandhya C, Sumantha A, Szakacs G, Pandey A. 2005. Comparative evaluation of neutral protease production by *Aspergillus oryzae* in submerged and solid-state fermentation. Process Biochemistry, 40:2689-2694.

Sarada I, Sridhar P. 1998.Nutritional improvement for Cephamycin C fermentation using a superior strain of *Streptomyces clavuligerus*. Process Biochemistry, 33:317-322.

Seyedeh LDK, Iran A, Vida M. 2011. Production of L-asparaginase from *Escherichia coli* ATCC 11303: Optimization by response surface methodology. Food and Bioproducts Processing, 89:315-321.

Umesh K, Shamsher S, Wamik A. 2007. Pharmacological and clinical evaluation of L-asparaginase in the treatment of Leukemia. Critical Reviews in Oncology/Hematology, 61:208-221.

Wriston JC, Yellin TO. 1973. L-asparaginase: a review. Advances in Enzymology-and Related Areas of Molecular Biology, 39:185-248.