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Paddy Straw: An Inexpensive Substrate for the Production of L-Glutaminase Using Native Strain *Aspergillus fumigates*

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ABSTRACT

The aim of the present work was to investigate the feasibility of paddy straw as a substrate for L-glutaminase production. *Aspergillus fumigatus* was selected and optimized for enzyme production in solid state fermentation using several agro industrial residues. Maximum enzyme activity was observed in paddy straw. Optimum pH and temperature for L-glutaminase activity were found to be 6 and 30 °C at 80 % moisture content. Both physico-chemical and nutritional parameters had played a significant role in the production of the enzyme, L-glutaminase. The enzyme production was found associated with the growth of the fungal culture. Thus the present study proved that the fungal strain used is highly potential and useful for industrial applications.

Key words: L-glutaminase, Optimisation, Solid state fermentation and *Aspergillus fumigatus*.

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INTRODUCTION

In recent years, L-glutaminase (L-glutamine amidohydrolase EC 3.5.1.2) has been studied due to their unique biotechnological versatility and their ability to catalyze a wide spectrum of bioconversion reactions of flavour compounds. Microbial glutaminases are more stable than plant and animal counterparts. In addition to it, they have also been detected in mammalian tissues where they are the major enzymes responsible for catabolic breakdown of glutamine [1, 2].

On an industrial scale, glutaminases are produced mainly by *Aspergillus* and *Trichoderma* sp. [3, 4, 5]. From an industrial point of view, filamentous fungi are particularly interesting as producers of glutaminase because they excrete substantially greater amounts of glutaminolytic enzymes into an extra cellular culture medium than bacteria or yeasts. Industrially important enzymes have traditionally been produced by submerged fermentation (SmF). But in recent years, SSF processes have been increasingly used for the production of these enzymes. Interesting fact in SSF which gained renewed interest from researchers in view of its economic and engineering advantages are inexpensive agro-industrial residues [6].

The technique of SSF involves the growth and metabolism of microorganisms on moist solids in the absence or near absence of any free-flowing water. These fermentation systems, which are closer to the natural habitats of microbes, may prove more efficient in producing certain enzymes and metabolites [7, 8]. Compared with submerged fermentation, SSF offers many advantages for the production of enzymes such as high productivity, simple technique, low production cost, less energy requirement, less wastewater production and better product recovery [9, 10, 11].

Moreover, the primary advantage of SSF is the fact that many metabolites are produced at higher concentration. And the crude fermented products from SSF can be used directly as the enzyme source for biosynthesis and biotransformation. There are also reports on the microbial production of extracellular L-glutaminase under SSF [12]. Keeping in view the extensive industrial importance of the enzyme, a study was carried out for optimization of fermentation parameters for L-glutaminase production by *Aspergillus fumigatus* using paddy straw.

MATERIALS AND METHODS

Fungal strain

The strain *Aspergillus fumigatus* used in this study was isolated from soil during a screening study for L-glutaminase producing microorganisms [13].The culture was maintained on sabouraud's dextrose agar (SDA) at 30 ℃ and sub-cultured every four weeks.

Chemicals

Chemicals were all of analytical grade. The agro industrial residues used in the study were purchased from the local market.

Inoculum preparation

The fungi were grown on SDA plates at 30 ± 2 °C. After profound sporulation, the plates were scrapped with 10 ml of sterile water containing 0.1% Tween-80 under aseptic condition. The spore suspension adjusted to desired count (10^6 spores/ml) using haemocytometer served as an inoculum.

L-glutaminase production in solid state fermentation

Five grams of agro industrial residue (wheat bran) were dispensed into 250 ml Erlenmeyer conical flasks and moistened with 10 ml of salt solution containing glucose 0.6%, KH_2PO_4 0.1%, $MgSO_4.7H_2O$ 0.05% and KCl 0.05%. The flasks were autoclaved at 121°C for 25 min, cooled to room temperature and inoculated with 2 ml of the fungal conidial suspension which prepared previously. The inoculated flasks were mixed thoroughly and incubated at 30 °C for 5 days. The crude L-glutaminase was extracted with 40 ml of the citrate phosphate buffer (pH 7.0), centrifuged at 4 °C for 20 min at 10,000 rpm.

L-glutaminase assay

Assay of glutaminase was carried out by slight modification of Imada using L-glutamine as substrate [14]. L-glutamine (1 ml of 1%) in 0.01 M phosphate citrate buffer (pH 7.0) was made to react with 1 ml of glutaminase enzyme for 60 min at 37 °C. The enzymatic activity was stopped by adding 0.5 ml of 1.5 M trichloroacetic acid. The reaction mixture was centrifuged at 5,000 rpm for 5 min to remove the precipitated protein. Then 0.1 ml of above mixture was taken and added to 3.7 ml of distilled water. Then 0.2 ml Nessler's reagent was added to it, after 15 min the developed color was measured at 480 nm using a spectrophotometer [15]. One unit (U) of L-glutaminase was defined as the amount of enzyme that liberates 1µ mol of ammonia under optimal assay conditions.

Optimisation

The strategy followed was to optimize each parameter (solid substrates, incubation period, initial moisture content, pH, temperature, carbon and nitrogen sources and metal ions), independent of the others and subsequently optimal conditions were employed in all experiments.

Selection of substrates: Bengal gram husk (BG), Black gram husk (BH), Coconut oil cake (COC), Corn cob (CC), Cotton shell (CS), Cotton seed (CO), Green gram husk (GH), Groundnut oil cake (GOC), Lemon peel (LP), Orange peel (OP), Paddy straw (PS), Pineapple waste (PW), Potato peel

(PP), Rice bran (RB), Rice straw (RS), Saw dust (SD), Soybean stover (SS), Sugarcane bagasse (SB), Wheat bran (WB) and Wheat straw (WS) were evaluated for L-glutaminase production.

Effect of incubation periods: Fermentation period was an important parameter for enzyme production by *Aspergillus fumigatus*. In this study, fermentation experiment was carried out up to 7 days and production rate was measured at 24 h regular intervals.

Effect of moisture content: To investigate the influence of the moisture content (before autoclaving) of the substrate, the fermentation was carried out under various moisture contents (10 to 90 %) of paddy straw which was adjusted with distilled water.

Effect of pH: The medium was adjusted using 0.1 M citrate phosphate for pH values between 3.0 and 7.0, in order to study the effect of pH on L-glutaminase production by *Aspergillus fumigatus*.

Effect of temperature: temperatures ranging from 25 to 50 °C were tested for their effect on L-glutaminase production.

Effect of various carbon and nitrogen sources: the effect of various carbon sources (sucrose, glucose, lactose, galactose and dextrose) were used for studying. Effects of various nitrogen sources (peptone, yeast extract, urea, casein and albumin) at a concentration of 1% w:v was studied.

Effect of metal ions: $CaCl_2$, KCl, $MgSO_4$, NaCl and $ZnSO_4$ at a concentration of 1% w:v were studied for their L-glutaminase production.

Statistical analysis

The statistical software, SPSS version 16.0 was used to estimate the statistical parameters. All statements of significance were calculated by One-way ANOVA, based on 95% confidence level.

RESULTS AND DISCUSSION

Selection of substrates

The potential economical advantages of solid state cultures may include a reduced thermal processing requirements, reduced energy requirement for agitation with high extracellular product concentration. Solid substrates employed in SSF processes are insoluble in water and act as a source of carbon, nitrogen, minerals as well as growth factors [16]. Results recorded in Figure 1 showed the maximum with an enzyme yield of 84 U/g when paddy straw was employed as a substrate. The selection of the ideal industrial waste for the enzyme production in a solid state fermentation process depends upon several factors mainly related with cost and availability of the substrate material and thus may involve screening of several

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agro-industrial residues [17]. The result in the present study indicated that L-glutaminase enzyme production pattern varied with agro-industrial residues.

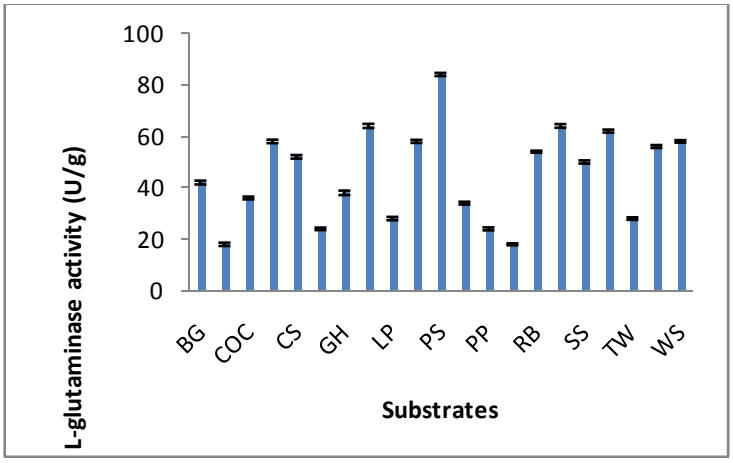

Figure 1: Effect of various substrates in L-glutaminase production

Effect of incubation periods

Maximum enzyme production could be obtained only after a certain incubation time which allows the culture to grow at a study state. Growth rate and enzyme synthesis of the culture are the two main characteristics which are mainly influenced by incubation time [18]. Enzyme production of each strain differs depending upon the specific growth rate of the strain. The maximum enzyme productivity (60 U/g) by the experimental fungal isolate was obtained at 5 days of cultivation is presented in Figure 2. Results of our study are in line with those produced by *Aspergillus oryzae* [19, 20].

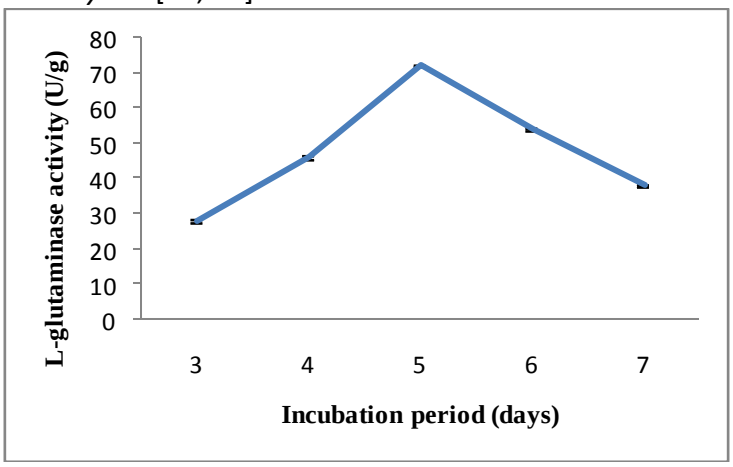

Figure 2: Effect of incubation period in L-glutaminase production

Effect of moisture content

Initial moisture content is a key factor in the L-glutaminase enzyme production. Data presented in Figure 3 indicated that maximum glutaminase production was obtained (64 U/g) when SSF was carried out at a moisture content of 80% (Figure 3). Moisture optimization can be

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used to regulate and to modify the metabolic activity of the microorganism [21]. This could be accomplished by the faster growth of microorganism at higher moisture content and the subsequent early initiation of the enzyme production.

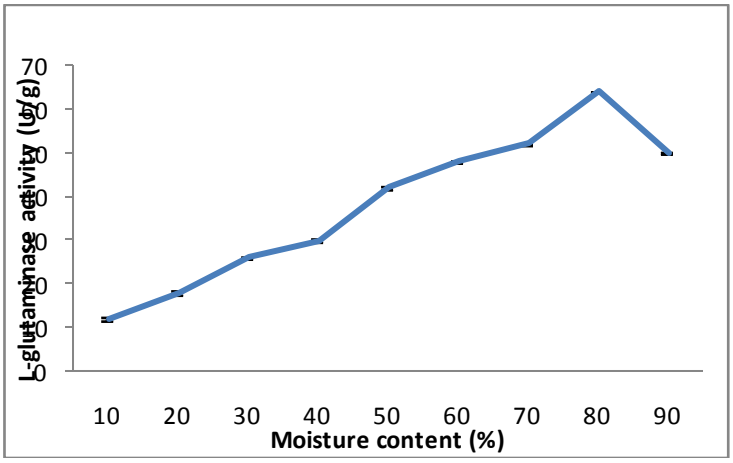

Figure 3: Effect of moisture content in L-glutaminase production

Effect of initial pH

Naturally a slight change in pH may affect the protein structure and a decline in enzyme activity beyond the optimum pH due to enzyme inactivation or instability. The pH ranging from 3 to 7 was studied for the detection of optimum pH. High glutaminase production was found at pH 6 (72 U/g) and minimum was observed at pH 3 (14 U/g) (Figure 4). These results are in coincidence with that reported for L-glutaminase production under solid state fermentation using *Trichoderma koningii* [5].

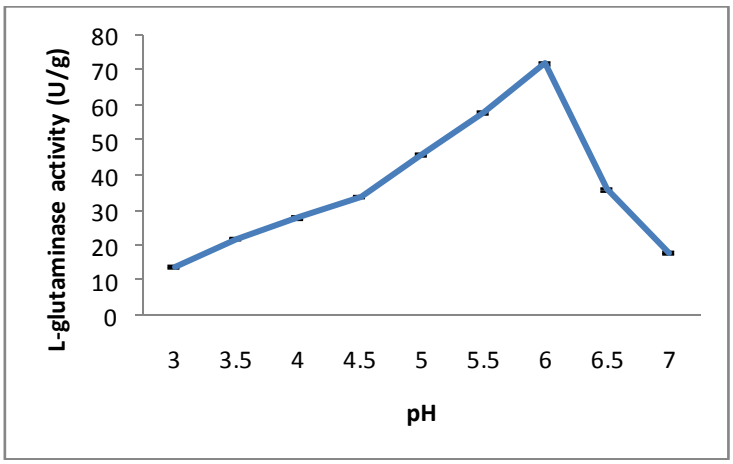

Figure 4: Effect of varying pH in L-glutaminase production

Effect of temperature

Temperature, a critical parameter has to be controlled which varies from organism to organism. It strongly affects the synthesis either non-specifically or specifically influencing the

rates of biochemical reactions. It can be observed from the Figure 5 that maximum yield (72 U/g) was obtained at 30 °C. In the present experiment with increase in temperature, enzyme production increased up to a certain level and upon further increase of temperature, production decreased. It might be due to the depletion of nutrients in the medium which stressed the fungal physiology resulting in the inactivation of secretary machinery of the enzymes. The optimum temperature of 30 °C obtained for L-glutaminase production by *Aspergillus fumigatus* is identical to that reported by El-Sayed in *Trichoderma koningii* [5].

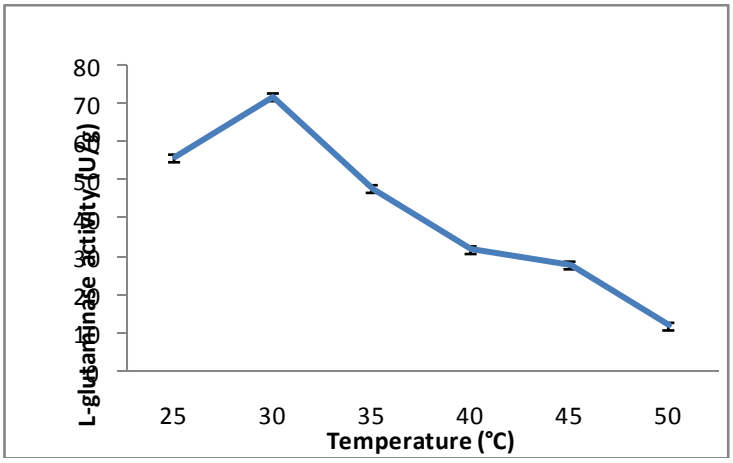

Figure 5: Effect of temperature in L-glutaminase production

Effect of various carbon and nitrogen sources

Carbon sources play a vital role in the cell metabolism and synthesis of glutaminase. The effect of carbon sources on the production of enzyme by *Trichoderma* sp. was investigated. Carbon sources tested for production of L-glutaminase enzyme showed maximum (48 U/g) when medium is incorporated with dextrose (Figure 6). Many researchers have studied the effect of additional carbon supplementation on the substrates. Sabu et al., reported maximum production on supplementation with glucose [12].

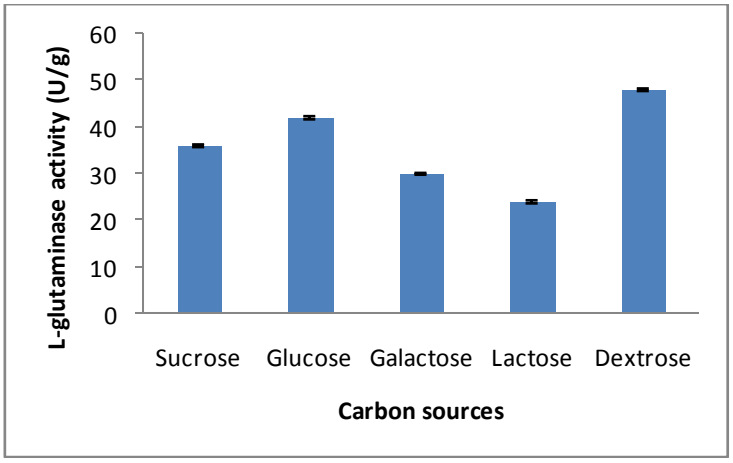

Figure 6: Effect of various carbon sources in L-glutaminase production

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The results on the effect of addition of nitrogen sources showed an impact on the production of L-glutaminase when yeast extract is incorporated into the fermentation medium. As presented in the Figure 7, high enzyme production was observed for yeast extract (58 U/g) followed by peptone (52 U/g). Evidently, except for lactose, all the compounds exerted a beneficial impact on glutaminase synthesis by the fungal culture. Similar results were reported for the production of L-glutaminase by the halophilic *Z. rouxii* under solid state fermentation [16].

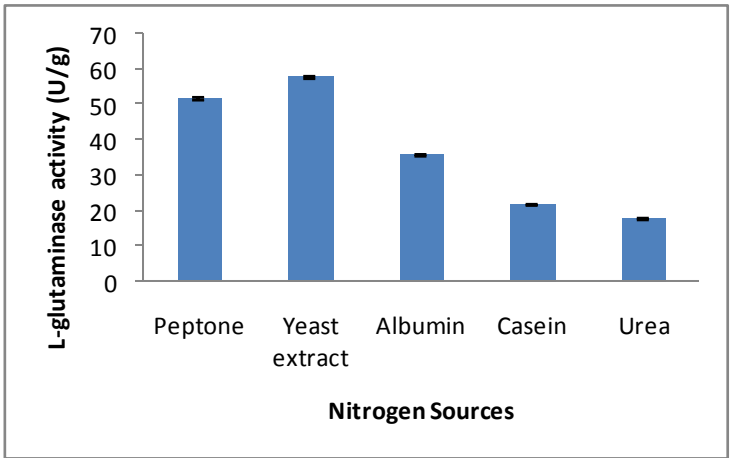

Figure 7: Effect of nitrogen substrates in L-glutaminase production

Effect of metal ions

When different metal ions were used in the solid state fermentation medium, the highest enzyme activity was obtained in NaCl (Figure 8) (52 U/g) and minimum at KCl (24 U/g). Hence the enzyme production could have resulted in better utilization of metal ions, which enhanced the L-glutaminase production. Similar results of inhibition of glutaminase activity by the addition of metal ions were reported by Prabhu and Chandrasekaran [22].

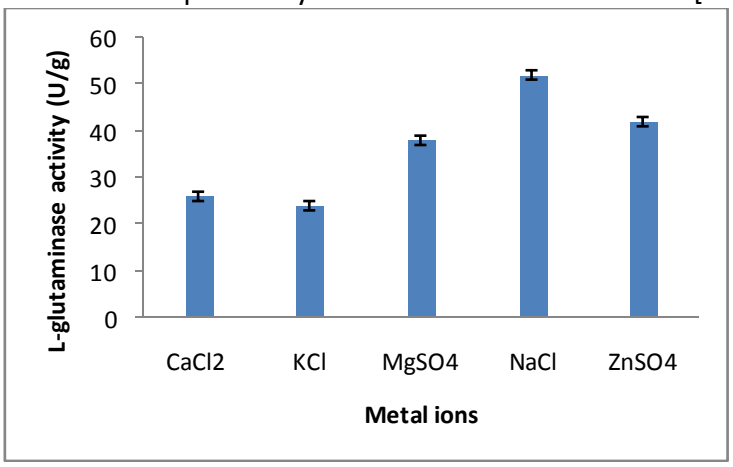

Figure 8: Effect of various metal ions in L-glutaminase production

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CONCLUSION

L-glutamine amidohydrolase is an industrially important enzyme which is mainly used in the food and pharmaceutical industry. As this enzyme broadens its wide applications, there is always a scope for novel glutaminase with better characteristics. From this study, we were able to establish that agro industrial wastes (paddy straw) which have not been exploited commercially for any industrial application and are poorly disposed could effectively be used as substrate for L-glutaminase production through the process of solid state fermentation.

REFERENCES

[1] Klein M, Kaltwasser H and Jahns T. FEMS Microbiol Lett 2002; 206: 63–67.
[2] Iyer P and Singhal RS. Biores Technol 2008; 99: 4300–4307.
[3] Tomita K, Yano T, Kumagai H and Tochikura T. J Ferment Technol 1998; 66: 299–304.
[4] Masuoa N, Itob K, Yoshimunea K, Hoshinoa M, Matsushimab H, Koyamab Y and Moriguchia M. Ptn Exp Pur 2004; 38: 272–278.
[5] El-Sayed ASA. Ind J Microbiol 2009; 4: 243–250.
[6] Khandeparkar RDS and Bhosle NB. Enz Micro Technol 2006; 39: 732–742.
[7] Mazutti M, Ceni G, Luccio MD and Treichel H. Bioproc Biosys Eng 2002; 30: 297–304.
[8] Peixoto-Nogueira SC, Sandrim VC, Guimara LHS, Jorge JAH, Terenz FI and Polizeli MLTM. Bioproc Biosys Eng 2008; 31: 329–334.
[9] Couto SR and Sanroman MA. J Food Eng 2006; 76: 291–302.
[10] Singhania RR, Patel AK, Soccol CR and Pandey A. Biochem Eng J 2009; 44:13–18.
[11] Sathya R, Pradeep BV, Angayarkanni J and Palaniswamy M. Biotechnol Bioproc Eng 2009; 14:788-794.
[12] Sabu A, Chandrasekaran M and Pandey A. Chem Today 2000; 18: 21–25.
[13] Nathiya K, Sooraj SN, Angayarkanni J and Palaniswamy M. Int J Phar Biosci 2011; 2: 297-302.
[14] Imada A, Igarasi S, Nakahama K and Isono M. J Gen Microbiol 1973; 76: 85–99.
[15] Wade H, Robinson HK and Philips BW. J Gen Microbiol 1971; 69: 99–312.
[16] Kashyap P, Sabu A, Pandey A, Szakacs G and Soccol CR. Proc Biochem 2002; 38: 307–312.
[17] Pandey A, Soccol CR, Rodriguez-Leon JA and Nigam P. Solid state fermentation in biotechnology, Asia Tech Inc, 2001, 237.
[18] Ellaiah P, Adinarayana K, Bhavani Y, Padmaja P and Srinivasulu B. Proc Biochem 2002; 38: 615-620.
[19] Koibuchi K, Nagasaki H, Yuasa A and Kataoka. J. Appl Microbiology and Biotechnology 2000; 54: 59–68.
[20] Yano T, Ito M, Tomita K, Kumagai H and Tochikura T. J Ferment Technol 1998; 66: 137–143.
[21] Pandey A. Solid-state fermentation an overview. In: Solid-state fermentation, New Delhi: Wiley, 1994, 3-10.
[22] Prabhu GN and Chandrasekaran M. J Mar Biotechnol 1996; 4: 176-179.