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PRODUCTION AND OPTIMIZATION OF L-ASPARGINASE FROM *ASPERGILLUS FLAVUS*

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ABSTRACT

The filamentous fungi isolated from paddy soil samples from Tiruvarur district.It was identified as *Aspergillus flavus* were screened for their ability to produce L-Asparaginase using modified Czapek Dox agar containing L-Asparaginase and phenol red as indicator. L-Asparaginase producing fungus isolated could be primilinary identified by plate assay method observing pink colour formation. It was found that *Aspergillus flavus* exhibited highest activity. The *Aspergillus flavus* were used for the optimization for the fermentation parameters like pH, temperature and spores size for L-Asparaginase production through solid state fermentation. The pH 8, temperature 30˚C and 1x107 spores/ml inoculums size were found optimum for maximum IU/ml of L-Asparaginase production.

KEYWORDS: L-Asparaginase, Czapek Dox agar, pH, temperature, and *Aspergillus flavus*

INTRODUCTION

Asparaginase (L-asparaginase amido hydrolase E.C.3.5.1.1) is an anti-neoplastic agent used in the lymphoblastic leukemia chemotheyrapy. Neoplastic cells cannot synthetase L-asparaginase due the absence of L-asparaginase synthetase (Keating *et al*., 1993).The L-asparaginase production using microbial systems has attracted considerable attention owning to the cost effective and eco- friendly nature. A wide range of microorganisms such as filamentous fungi, yeast and bacteria have proved to be beneficial source of these enzymes (Sarquis e*t al.,* 2004).

L-asparaginase catalyzes the hydrolysis of L-asparaginase into L-asparagic acid and ammonia as the several types of tumor cells requires (Fornier *et al.,* 2013). L-asparaginase is an essential amino acid for protein systhesis, they are derived of an essential growth factor in the presence of L-asparaginase (Shah *et al.*, 2010). It has received increased attention in recent years for its anticarcinogenic potential. The clinical action of this enzyme is attributed to the reduction of L-asparaginase, since tumor cells unable to synthesize this amino acid are selectively killed by L-asparaginase deprivation (Theantana *et al*., 2007). The enzyme present in many animal tissues, bacteria, plants and in the serum of certain rodents but not in mankind whereas microbial L-asparaginase has attracted considerable attention (El-Bessoumy *et al.,* 2004; Siddalingeshwara and Lingappa, 2011).

The enzyme for use as a drug is an important accent of today's pharmaceutical industry. L-asparaginase has been used an anti-tumor agent for the effective treatment of acute lymphoblastic leukemia and lymphosarcoma. It destroys asparagines external to the cell by hydrolysis into asparatic acid and ammonia. Hence normal cells are able to make all the asparagine they need internally whereas tumor cells become depleted rapidly and die. It is also known as Elspar, kidrolase, Leunase, Colaspase and Crasnitin (Basker *et al.*, 2010).

L-asparaginase belongs to an amidase group that produces aspartic acid and ammonia by asparaginase hydrolysis (Wriston and Yellin1973; Capizzi *et al.*, 1984). The important application of the L-asparaginase enzyme is in the treatment of acute lymphoblastic leukemia mainly children. Hodgkin disease, acute myelocytic leukemia, acute myelomonocytic leukemia, chronic lymphocytic leukemia, lymphosarcoma treatment, reticulosarbom and melanosarcoma (Stecher *et al.,* 1999; Verma *et al*., 2007). The role of L-asparaginase in lymphocytic leukemia cells treatment is based on the fact these cells are not capable of synthesizing L-asparagine and they rely on the exogenous source to get hold of L-asparaginase (Lee *et al.,* 1989).

The aim of the present study is to evaluate the optimization of fermentation factors like particle size temperature and P^H for the production of L-asparaginase employing locally isolated strain *Aspergillus flavus.*

MATERIALS AND METHODS

Isolation of fungi

The soil sample was collected from Mannargudi, Tiruvarur district, Tamil Nadu. Fungal strains were isolated on potato dextrose agar (PDA) after serial dilution was made. The inoculated agar plates were incubated at 28˚C for 2 to 5 days. Ten isolates of *Aspergillus flavus* were selected and tentatively identified in the laboratory as described by Rapper and Fennell and were maintained on potato dextrose agar at 4˚C. Further confirmation was done at Muthaiya Research Foundation for Biological Science, Thanjavur.

Screening of L-asparaginase

The methodology was based on Gulati *et al*., (1997) with the incorporation of phenyl red in a stock solution prepared in ethanol with L-Asparagine incorporated in the medium for the selection of the microorganism with the ability to produce L-Asparaginase.

Production of L- Asparaginase

The enzyme was produced by the methodology based on (Gulati *et al.,* 1997). The composition of the 1000ml of the production was glucose 2g, L asparagine 10g, K_2HPO_4 1.52g, KCl 0.52g, $MgSO_4.7H_2O$ 0.52g, $CuNO_3.3H_2O.7H_2O$ trace amount, $ZnSO_4.7H_2O$ trace amount and FeSO4 trace amount. The *Aspergillus flavus* was inoculated into sterilized medium and incubated at 28^0C for 8 days. After incubation period, 100ml of distilled water was added the mixture was shaken for 30 minutes at 37^0C to facilitate the extraction of the enzyme from the fermentation. The suspension was filtered through a whatman No: 1 filter paper and used as a crude enzyme.

Ezyme Assay for Asparaginase

0.5ml of crude enzyme was transferred to test tube containing 0.5 ml acetate buffer (pH-5.9) 0.5ml enzyme and 0.5ml distilled water to a total volume of 2.0ml was incubated at 28˚C for 3minutes. The reaction was terminated by adding 0.5ml of 1.5M trichloroacetic acid. Blank tubes were run by adding the enzyme preparation after the addition of trichloroacidic acid. Then 8.7 ml distilled water, 0.1ml of the above the mixture and 0.2ml Nessler's reagent were added. After keeping the mixture at 15 to 28˚C for 20 min, OD was taken at 450nm with a spectronic 20 colorimeter (Imade *et al.,* 1973).

Optimization of pH for L-asparaginase production

The fermentation medium was adjusted with different pH's 4, 5, 6, 7& 8

were used for the determining the influence of pH on enzyme production by *A. flavus*.

Optimization of temperature for L-asparaginase production

The fermentation medium was adjusted with different temperatures 20, 25, 30, 35 & 40^0C were used for the determining the influence of temperature on enzyme production by *A. flavus*.

RESULT AND DISCUSSION

In the present study fungi such as *Aspergillus sparsusi, A. janthinellum, A.ochraceous, A. flavus and A. terreus* were isolated from the paddy soil. Of these *A. flavus* was dominantly presented, so it was selected as potential strain for the production of L-asparaginase.

The media optimization is an important aspect to be considered in the development of fermentation considered in the development of fermentation technology. However, there are only a few reports concerning the optimization of media composition especially for fungal strains in enzyme production. The incubation period varies with enzyme production (Smitt *et al*., 1996). Short incubation period offers potential for inexpensive production of enzyme (Sonjoy, Bill Bex and Houston., 1995). In the present study the Asparaginase activity increased steadily and reached maximum at 96 hours of incubation. This result was similar to Mishra, who reported the highest L-asparaginase activity of *A.niger* in medium the optimal period for enzyme production was 96 hours.

The fermentation medium was adjusted with different pH's 4, 5, 6, 7& 8 were used for the determining the influence of pH on enzyme production by *A. flavus*. The observed results in the present study are the optimum pH was found to be pH 7 (Fig. 1). De Angeli *et al* 1970 and Ali *et al.* 1994 have reported pH 7 and 4.5 were optimum for the maximum production of L-asparaginase and SmF process respectively. Gulati *et al.*, have reported 6.2 was the optimum pH for L-asparaginase production by *A. terreus* strains. Similarly, Sarquis *et al.*, have reported highest L-asparaginase production of 58U/L when *Aspergillus* strains cultivated in medium having pH of 6.2. In our study the data revealed that the pH of 7.0 was found as suitable for maximum production of L-asparaginase with *A.flavus*.

The external temperature shows a significant effect on the cell growth metabolism and there by the production of L-asparaginase. In this present study, the production medium was adjusted with different temperatures 20, 25, 30, 35, and 40^0C. A maximum production of l-asparaginase by *A. flavus* was found to increase with temperature upto 30°C (Fig. 2) and production reduced at temperature higher than 30 ^{0}C. The temperature normally employed in the range of 20-40 ^{0}C and it

depends mostly on the growth kinetics of the microorganisms. L-asparaginase showed maximal activity when assayed at a P^H -8 on *Aspergillus flavus*. The activity of L-asparaginase was estimated in different interrals at (4-8). The maximum amount of enzyme production was observed in P^H -7 (6.33IU/ml).

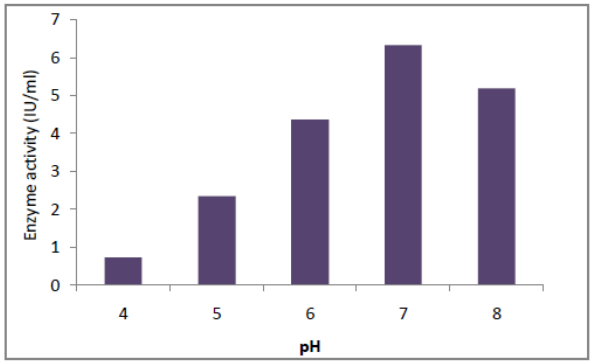

Fig. 1 Effect of pH on L-asparaginase production

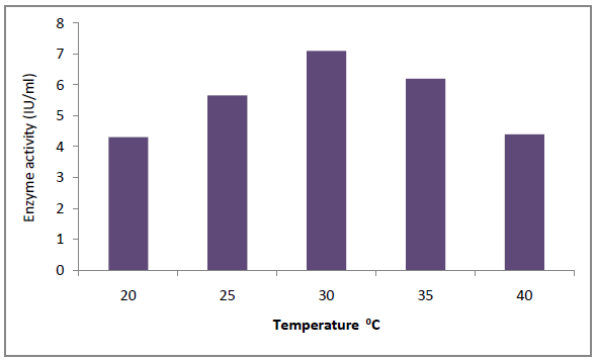

Fig. 2 Effect of temperature on L-asparaginase production

The screening of an ideal agro-residual based substrate for maximum enzyme production in a solid-state fermentation process mainly depends upon its easier degradation into nutrients and uptake by the microorganism to synthesize the targeted metabolite, its cost effectiveness and availability in the nature. The present study revealed that L-asparaginase production pattern varied with the type of agro-residual substrates. This could be accredited to solid materials dual role-supply of nutrients to the microbial culture for its growth and anchorage for the growing cells. Maximum enzyme production (11.5 U/gds) was observed with wheat bran, while minimum L-asparaginase production (7.2 U/gds) was noticed with rice husk as substrate/support material. Wheat bran contains approximately 18% protein, 5% fat and 62% carbohydrate (Madruga et al., 2000) and is rather complete source of nutrients for microorganisms (Ellaiah *et al*., 2004; Beg *et al.*, 2000).

In conclusion, several fungal strains were isolated from soil and were capable of L-asparaginase production. Among these *Aspergillus flavus* was found to produce the highest L-asparaginase activity of 7.11IU/ml after optimization with pH and temperatures.

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