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Research Article

PRODUCTION AND IMMOBILIZATION OF L-ASPARAGINASE FROM MARINE SOURCE

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ABSTRACT

Enzymes are proteins that catalyze (*i.e.*, increase the rates of) chemical reactions. The manufacture of an enzyme for use as a drug is an important facet of today's pharmaceutical industry. Microbial L-Asparaginase (L-Asparaginase amido hydrolase) has been widely used as a therapeutic agent in the treatment of certain human cancers, mainly in acute lymphoblastic leukemia. Thermophilic fungi can be grown in minimal media with metabolic rates and growth yields comparable to those of mesophilic fungi. L-asparaginase perform essential role in the treatment of acute lymphoblastic leukemia, lymphosarcoma and in many other clinical experiments relating to tumour therapy in combination with chemotherapy. Fungal isolates were isolated from soil samples collected from different regions of the Arabian Sea, using potato dextrose agar (PDA) medium by serial dilution method. The inoculated agar plates were incubated at 37°C for 4 to 7 days. Twelve isolates w ere selected and the isolated strains were screened by plate assay method using Czpak Dox medium and potential strains were used for the production of L-Asparaginase. It was found out that among the twelve isolates five showed significant production. One IU of L-Asparaginase is the amount of enzyme which liberates 1µmol of ammonia per minute per ml [µmole/ml/min]. From this work we conclude that more than 80% of the fungal strains from marine soil sample had the ability to produce the enzyme L-Asparaginase. The highest immobilized activity and highest immobilization yield were obtained with Silica gel carrier.

Keywords: L- Asparaginase, Thermophilic fungi, Lymphoblastic leukemia, Immobilization.

INTRODUCTION

Enzymes are proteins that catalyze (*i.e.*, increase the rates of) chemical reactions. In enzymatic reactions, the molecules at the beginning of the process are called substrates, and they are converted into different molecules, called the products. Almost all processes in a biological cell need enzymes to occur at significant rates. Since enzymes are selective for their substrates and speed up only a few reactions from among many possibilities, the set of enzymes made in a cell determines which metabolic pathways occur in that cell. The manufacture of an enzyme for use as a drug is an important facet of today's pharmaceutical industry.

Microbial L-Asparaginase (L-Asparaginase amido hydrolase, E.C.3.5.1.1) has been widely used as a therapeutic agent in the treatment of certain human cancers, mainly in acute lymphoblastic leukemia. The discovery of L-asparaginase, a medical agent for the treatment of malignant tumors, was made in1922. Clementi showed that guinea pig serum contained a high activity of L-asparaginase. L-asparaginase catalyses the conversion of l-asparagine to l-aspartate and ammonium, and this catalytic reaction is essentially irreversible under physiological conditions. Supplementation of L-asparaginase results in continuous depletion of l-asparagine. Under such an environment, cancerous cells do not survive. This phenomenal behavior of cancerous cells was exploited by the scientific community to treat neoplasias using L-asparaginase.

This enzyme is also a choice for acute lymphoblastic leukemia, lymphosarcoma and in many other clinical experiments relating to tumour therapy in combination with chemotherapy. This treatment brought a major breakthrough in modern oncology, as it induces complete remission in over 90% of children within 4 weeks. With the development of its new functions, a great demand for L-asparaginase is expected in the coming years.

This enzyme is widely distributed being found in animal, microbial and plant sources. It has been observed that eukaryotic microorganisms like yeast and fungi have a potential for asparaginase production.

Immobilization of enzymes on natural biopolymers, such as grafted carrageenan, chitosan and alginate will, practically, enable the separation and reusability of the enzymes for tens of times, which will, consequently, reduce both the enzyme and the product costs. Accordingly, we are expecting the prices of foods and drugs to be significantly reduced. One of the major advantages of the immobilization of enzymes, that we have achieved, is the improvement of the enzymes' thermal, as well as, operational stabilities.

MATERIALS AND METHODS

Collection of Samples

Fungal isolates were isolated from soil samples collected from different regions of the Arabian Sea, using potato dextrose agar (PDA) medium by serial dilution method. The inoculated agar plates were incubated at 28°C for 4 to 7 days. Twelve isolates were selected and tentatively identified in the laboratory as described by Rapper and Fennell, and were maintained on potato dextrose agar (PDA) at 4°C.For further characterization the isolates were sent to Madras University, Chennai.

Production of l-Asparaginase

The isolated strains were screened by plate assay method using Czepak Dox medium and potential strains were used for the production of L-Asparaginase. It was found out that among the twelve isolates five showed significant production.

Fermentation Studies

The production of L-Asparaginase was carried out by using fermentation process using Asparagine. The moisture content of the flask is 65% were maintained and inoculated 1 ml of inoculums (1×10^7spores/ml). The content of the flask were mixed thoroughly gently beating the flask on the palm of the hand and incubated in a shaker incubator at 37°C for 5 days. The pH5 was maintained throughout the fermentation process.

Extraction of Fermented Substrate

The samples were withdrawn periodically at 24hrs in aseptic condition 1gm of moldy substrate was taken into a beaker and distilled water was added to it. The contents of flasks were allowed to have contact with water for 1 hr with occasional stirring with a glass rod. The extract was filtered through Whatman filter No.1. The clear extract was centrifuged. The supernatant were used as enzyme preparation. Thus prepared crude enzyme was used for assay.

Quantitative Assay for L-Asparaginase Activity

Assay of enzyme was carried out as per Imad et al.[18]. 0.5ml of 0.04M asparagine was taken in a test tube, to which 0.5ml of 0.5 M buffer

(acetate buffer pH-5.4). 0.5ml of enzyme and 0.5ml of distilled water was added to make up the volume up to 2.0ml and incubate the reaction mixture for 30min. After the incubation period the reaction was stopped by adding 0.5ml of 1.5M TCA (Trichloracetic acid). 0.1ml was taken from the above reaction mixture and added to 3.7ml distilled water and to that 0.2ml Nessler's reagent was added and incubated for 15 to 20 min. The OD was measured at 450 nm. The blank was run by adding enzyme preparation after the addition of TCA. The enzyme activity was expressed in International unit.

International Unit (IU)

One IU of L-Asparaginase is the amount of enzyme which liberates 1µmol of ammonia per minute per ml [µmole/ml/min].

Immobilization of L- Asparaginase

Different supports were employeed for L- asparaginase immobilization according to Experimentally, 200 mg of each supports was shaken in 5ml tris-Hcl buffer (0.01 mole. pH 8.6) containing 2.5% glutaraldehyde at room temperature for 2 hrs. The carries were filtered off and washed with distilled water to remove the excess of glutaraldehyde then each treated carrier was incubated with 5 ml of tris- HCl buffer containing 1ml of enzyme. After being shaken for 2 hrs at 30^0 C. the unbounded enzyme was removed by washing with distilled water until no protein or activity were detected in the wash.

RESULTS AND DISCUSSION

Twelve fungal isolates from different soils in Arabian Sea region were isolated and screened for L-Asparaginase activity. The potential strains were selected on the basis of pink zone around the colony by plate assay method. Out of twelve strains five were selected as potential strain for the production of L-Asparaginase.

From this five potential strains, S_4 exhibited higher zone of diameter and considered as potential strain for L-Asparaginase production among the strains isolated from the soil. As such, strain S3, S5 can be treated as moderate L- Asparaginase producers and remaining isolates treated as poor L-Asparaginase producers.

Production of L-asparaginase

The five were selected as potential strain for the production of L-Asparaginase through solid state fermentation. The fermentation studies were indicated that the L-asparaginase production was maximum at 48 hrs fermentation period.

They were examined for L- asparaginase production. Among these, five isolates showed pink colour on modified czepak dox's medium containing phenol red as indicator, indicating the increase in pH which originated from ammonia accumulation in the medium. The dye indicator is yellow at acidic condition and turns to pink at alkaline condition (figure.1). Since this method is very simple and rapid for the detection of L-asparaginase activity, it has been used for L- asparaginase production from thermophilic fungi isolated from marine soil sources.

From this five potential strains, S_4 exhibited higher production and considered as potential strain for L- asparaginase production among the strains isolated from the soil. As such, strain S_3, S_5 can be treated as moderate L- asparaginase producers and remaining isolates treated as poor L-Asparaginase producers.

Fig. 1: Production of L- asparaginase

L- asparaginase activity in normal condition

The analysis of L-asparaginase production was carried out at every 24 hours interval after inoculation with the maximum activity obtained at 48 hours of incubation (figure -2). The yield of L-asparaginase increased with the increase in the initial pH of the medium up to 7.0 and thereafter it decreased. The enzyme L- asparaginase was assayed by estimating the amount of ammonia released in the reaction. The amount of Ammonia released by the test sample was calculated with reference standard graph. (Soni, K., *et al.,*1989).

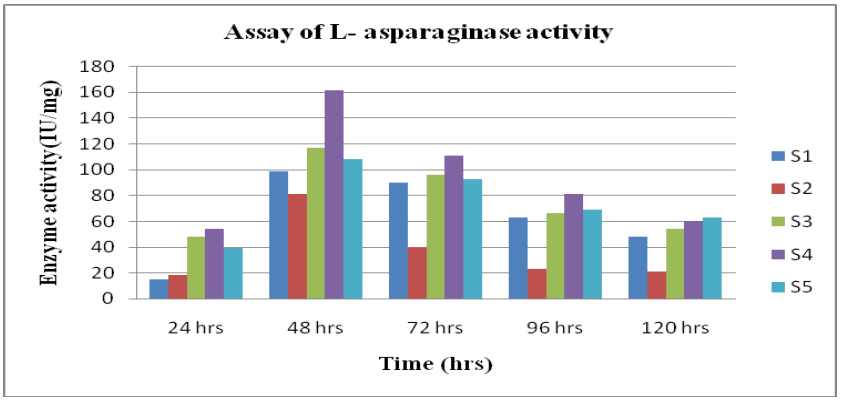

Fig. 2: Assay of L-asparaginase activity

Immobilization of L- asparaginase

Two supports, pre-activated with glutaraldehyde, react with different degrees with the terminal amino residues of the enzyme protein. Immobilization of L-asparaginase by covalent binding was achieved by cross linking between the enzyme and different carriers (i.e. silica gel, agarose, activated carbon, celite, carboxymethyl cellulose, egg shell, tricalcium phosphate) throughout glutaraldehyde. The amount of enzyme used for immobilization was 1ml carrier. The result indicated that the lowest immobilized activity and immobilized yield carrier and 40.0, 27.3, 38.0, 60.0, and 51.2 % were detected with agarose carrier.

On other hand, the highest immobilized activity and highest immobilization yield 45.24, 32.56, 50.00, 86.84 and 71.79 % were obtained with Silica gel. The results are shown in (fig.3&4)

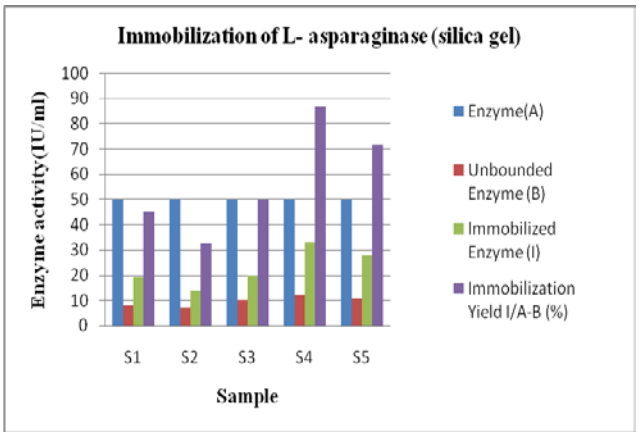

Fig. 3: Immobilization of L- asparaginase (Silica gel carrier)

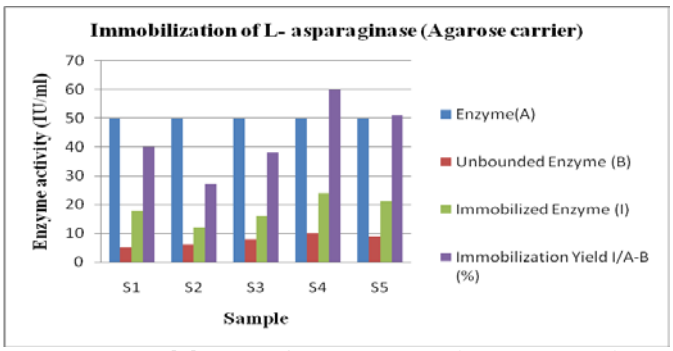

Fig. 4: Immobilization of L- asparaginase (Agarose carrier)

SUMMARY AND CONCLUSION

Microbial L-Asparaginase has been widely used as a therapeutic agent in the treatment of certain human cancers. With the development of its new functions a great demand for L-asparaginase is expected in the coming years.

Twelve isolates were taken from different soil samples of the Arabian Sea region and screened for L-Asparaginase activity. Five isolates exhibited significant production of L-Asparaginase by plate assay method.

Immobilization of L-asparaginase by covalent binding was achieved by cross linking between the enzyme and different carriers throughout glutaraldehyde. The result indicated that the lowest immobilized activity and immobilized yield were obtained with agarose carrier. On other hand, the highest immobilized activity and highest immobilization yield were obtained with Silica gel carrier.

From this work we conclude that more than 80% of the fungal strains from marine soil sample had the ability to produce the enzyme L-Asparaginase.

In future, isolated fungus will be taken to categorize its genus, species and also to optimize enzyme production.

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