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ISOLATION, SCREENING AND PRODUCTION OF L-ASPARAGINASE BY THERMOPHILIC FUNGI FROM MARINE SOIL SOURCE

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ABSTRACT

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. 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. 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. 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 were selected and the isolated strains were screened by plate assay method using Czepk Dos 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. It was also found out from the present study that the sample MS_4 at 30˚C in pH6 showed maximum activity. In future, isolated fungus will be taken to categorize its genus, species and also to optimize enzyme production.

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[1,2].

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[3,4]. 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[5].

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[6,7]. 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[8].

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[9].

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 Czepk Dos 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.

Optimization of fermentation parameters for L-Asparaginase production:

The production of L-Asparaginase mainly depends on factors like temperature and ph. Hence these parameters must be optimized in order to achieve higher yields of L-Asparaginase. During

this optimization process, once a particular parameter was optimized, the same optimum condition of that specific parameter was employed in the subsequent studies wherein another parameter is to be optimized.

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 450nm. 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].

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, MS_4 (Table.1) exhibited higher zone of diameter and considered as potential strain for L-Asparaginase production among the strains isolated from the soil. As such, strain MS3, MS5 can be treated as moderate L- Asparaginase producers and remaining isolates treated as poor L-Asparaginase producers.
The effects of different pH (4,6) (Table.2 &3) and temperature on L-Asparaginase production revealed that the yield of L-Asparaginase increased with the increase in initial pH of the substrate up to 6 units at 30°C. These increasing peaks were observed up to 72 hours of fermentation period. No significant production of L-Asparaginase was found in pH 4 at 37°C.

Table 1 L-ASPARAGINASE ACTIVITY IN NORMAL CONDITION

Sample	24 Hrs	48 Hrs	72 Hrs	96 Hrs	120Hrs
MS1	0.3078	0.9233	1.3441	1.2971	1.0090
MS2	0.1032	0.1737	1.8755	1.5494	1.0983
MS3	0.1034	0.2825	1.5286	1.2598	0.9686
MS4	0.3704	0.8619	1.9999	1.4501	1.0956
MS5	0.2008	0.6563	1.4485	1.2456	1.0974

Table.2 L-ASPARAGINASE ACTIVITY AT 30˚C WITH pH 4

Sample	24 Hrs	48 Hrs	72 Hrs	96 Hrs	120 Hrs
MS1	0.0681	0.3159	1.3835	0.7299	0.3010
MS2	0.0999	0.3999	1.3737	1.0978	0.2850
MS3	0.0509	0.3408	1.7803	0.9327	0.4535
MS4	0.1094	0.3477	1.8759	1.5321	1.3370
MS_5	0.0609	0.3542	1.6154	1.3021	1.2450

Table 3 L-ASPARAGINASE ACTIVITY AT 30˚C WITH pH 6

Sample	24 Hrs	48 Hrs	72 Hrs	96 Hrs	120 Hrs
MS1	0.0349	0.2188	1.0935	1.3369	0.3317
MS2	0.0392	0.1963	1.5034	0.7134	0.1669
MS3	0.0437	0.2743	1.8879	1.3387	1.0935
MS4	0.2078	0.3308	2.1279	1.6161	1.1576
MS5	0.0243	0.1634	1.3512	1.3060	0.1985

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.

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. It was also found out from the present study that the sample MS_4 at 30˚C in pH6 showed maximum activity. In future, isolated fungus will be taken to categorize its genus, species and also to optimize enzyme production.

REFERENCES

1. Ali S.S., Micropial L-asparaginase and their activities, Final Technical Report DST New Delhi, 1989.
2. Krasotkina J, Anna A, Borisova Yuri V, Gervaziev and Nikolay N Sokolov., One step purification and kinetic properties of the recombinant L- asparaginase from *Erwinia caratovora*, Biotechnol Appl Biochem, 2004; 39:215-22.
3. Swain A.L, Jaskolski M, Housset D, Mohana Rao J.K, Wlodawer A., Crystal structure of Escherichia coli L-asparaginase, an enzyme used in cancer therapy, Proc Natl Acad Sci, 1993; 90:1474-1478
4. Mannan S, Sinha A, Sadhukhan R, Chakrabarty S.L., Purification, characterization and antitumor activity of L-asparaginase isolated from *Pseudomonas stutzeri* MB-405, Curr Microbiol, 1995; 30:291-298.
5. Sarquis M.I.M, Oliviera E.M.M, Santos A.S and Da-Costa G.L., Production of L-asparaginase by filamentous fungi, Mem Inst Oswaldo Cruz, 2004; 99:489-492.
6. Elzainy T.A and Ali T.H., Detection of the antitumor glutminase-asparaginase in the filamentous fungi, J Appl Sci, 2006; 6:1389-1395.
7. Ferrara M.A, Severino N.M.B, Mansure J.J, Martin A.S, Oliveira E.M.M, Siani A.C, Torres F.A.G and Bon E.P.S., Asparaginase production by a recombinant *Pichia pastoris* strain harbouring *Saccharomyces cerevisiae ASP3* Gene, Enz Microbial Technol., 2006; 39: 1457-1463.
8. Borkotaky B, and Benzbaruah R.L., Production and properties of asparaginase from a new *Erwinia* sp, Folia Microbial, 2002; 47: 473-476.
9. Prakashan R.S, Rao C.S, Rao R.S, Lakshmi G.S and Sarma P.N., L-Asparaginase production isolated *Staphylococcus* sp.-6A: design of experiment considering interaction effect for process parameter optimization, J Appl Microbial, 2007; 102: 1382-1391.