SJIF Impact Factor: 5.464

WORLD JOURNAL OF ADVANCE HEALTHCARE RESEARCH

ISSN: 2457-0400
Volume: 6.
Issue: 5.
Page N. 162-165
Year: 2022

Original Article www.wjahr.com

TUMOR INHIBITORY L-ASPARAGINASE PRODUCTION

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Received date: 26 February 2022 **Revised date:** 19 March 2022 **Accepted date:** 08 April 2022

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ABSTRACT

The present research work demonstrates the production of L-asparaginase using *Neolamarckia cadamba* as substrate by *Streptomyces ambofaciens* in a solid state fermentation. Generally L-Asparaginase enzyme was present in many animal tissues, plant tissues and bacterial cell, but not in human cell. L-asparaginase is a chemotherapeutic agent plays a vital role in treatment of lymphosarcoma, lymphoproliferative disorders and acute lymphocytic leukemia**.** In this research, L-asparaginase enzyme production parameters like incubation time, incubation temperature, pH, inoculum level and moisture content were optimized. Different carbon supplements were screened for their influence on enzyme activity; they are sucrose, maltose, glucose, fructose, and lactose. The fermentation time of 48hrs, the temperature of 32^0C, pH 6.0, inoculum level of 80%v/w and moisture content of 70%v/w were observed optimum for the production of L-asparginase. Among carbon supplements sucrose gave better yield. L-asparagine of 0.3% w/w as nitrogen source was observed optimum for the production of L-asparaginase. Final conclusion that *Neolamarckia cadamba* could be a promising agent for industrial application since it produces a significant L–asparaginase (85.66 U/ml) activity in solid state fermentation.

KEYWORDS: *Neolamarckia cadamba, Streptomyces ambofaciens*, Solid-state fermentation.

INTRODUCTION

Solid-state fermentation is a process that occurs on a non-soluble material that acts both as support and a source of nutrients, with a reduced among of water, under the action of fermenting agent.[1] A large amount of researches has been conducting on the biosynthesis of Lasparaginase[2] demonstrated anticancer activity. L-asparaginase was produced throughout the world by Solid-state fermentation (SSF) and submerged fermentation (SF). This SF technique has many disadvantages, such as the low concentration production, and consequent handling, reduction, and disposal of large volumes of water during the downstream processing. Therefore, the SF technique is a cost intensive, highly problematic, and poorly understood unit operation.[3] Solid-state fermentation is a very effective technique as the yield of the product is many times higher when compared to that in SF.[4] L-Asparaginase (E. C. 3. 5. 1. 1) is present in many animal tissues, bacteria and plants, but not in mankind. Microbial asparaginases have been particularly studied for their applications as therapeutic agents in the treatment of certain types of human cancer.[5] L-asparaginase from two bacterial sources (*E. coli* and *Erwinia carotovora*) is currently in clinical use for the treatment of acute lymphoblastic leukemia.[6] It is also used for the treatment of various carcinoma[7] and bovine lymphomosarcoma.[8] Therefore, the aim of the present research work is the discovery of a new L-asparaginase producer that is serologically different from the previously reported ones, but one that has similar therapeutic effects.

MATERIAL AND METHODS

Substrate: *Neolamarckia cadamba* was collected from our college garden, sathupally and dried naturally and powdered, packed and stored until further use.

Microorganism: *Streptomyces ambofaciens* was used for the production of L-asparginase enzyme using *Neolamarckia cadamba* as substrate. Nutrient agar medium was used for sub culturing and maintenance of the microorganism.

Preparation of Inoculum: Streaking are done from the old cultures of *Streptomyces ambofaciens* on pure nutrient agar medium and incubate them at 30^0C for 3

days.

Development of Inoculum: 10ml of sterile distilled water were added to 3 days old agar slant; from that 1ml of suspension that contains approximately, 10^7cells/ml was used as the inoculums.

Solid State Fermentation: SSF was carried out in 250-mL flat bottom shallow glass container by taking production medium containing (in g/L): Glucose- 12.5g, NH_4NO_3- 2.66g, $FeSO_4.7H_2O$ - 0.01g, L-asparagine - 0.5g, KCl- 0.5g, K_2HPO_4-1g. The pH was adjusted to 6.8. SSF was carried out by taking 5g of substrate in 250ml Erlenmeyer flask, moistening it with 2.5ml of production medium, mixed thoroughly and autoclaved at 15lb pressure, 121^0C for 15min. After cooling, the flasks were inoculated with 1ml of cell suspension and incubated.

Determination of Enzyme Activity

Enzyme Extraction: The cultivation was carried out at a temperature of 30^0C for 24hrs interval. The solid state fermented material corresponding to one Erlenmeyer flask was mixed with 40ml of 0.1M Phosphate buffer and homogenized with constant stirring for 30min at 150rpm on rotary shaker, to extract the liquid from bacterial cells. That extract was filtered through whatmann filter paper and was centrifuged at 8000 rpm for 15 min.[9-10]

Enzyme Assay: L-asparaginase enzyme activity was determined by measuring the amount of ammonia formed by nesslerization. 0.5 mL sample of crude enzyme, 1.0 mL of 0.1M sodium borate buffer (pH 8.5) and 0.5 mL of 0.04M L-asparagine solution were mixed well and incubated for 10 min at 37^0C. The reaction was then stopped by the addition of 0.5 mL of 15% trichloroacetic acid and it was centrifuged, from this 1ml of supernatant liquid is collected and to it 1ml of Nessler's reagent was added. The liberated ammonia was measured by direct nesslerization. The yellow color was read in a spectrophotometer at 500nm. One unit (U) of L-asparaginase was the amount of enzyme which liberates 1µmole of ammonia in 1 min at 37^0C .

RESULTS AND DISCUSSION

To determine the effect of incubation time on enzyme production, the medium incubate at different time intervals and the maximum L-asparaginase activity was observed at 48hrs. After 48hrs, it was decreased due to depletion of nutrient materials. L-asparaginase production at different time intervals is shown in the fig.1.

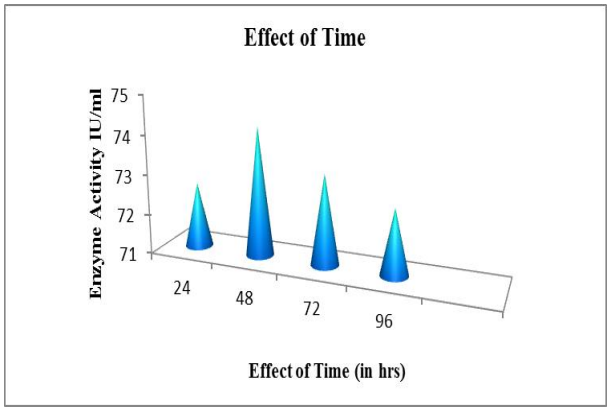

Fig.1: Effect of time on enzyme production.

The temperature is very critical in SSF as it ultimately affects the growth of the microorganism. The maximum amount of L-asparaginase was observed at 32^0c temperature Fig.2.

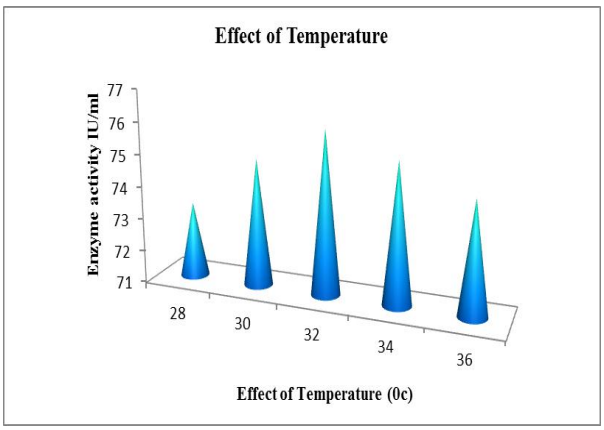

Fig.2: Effect of temperature on enzyme production.

To determine the effect of pH, the bacterial nutrient medium was adjusted with different pH ranges 4, 5, 6, 7 and 8.0. The maximum production of L-asparaginase was recorded at pH 6.0 fig.3.

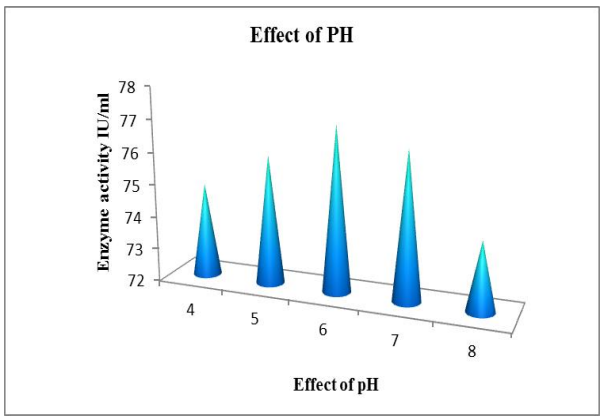

Fig.3: Effect of pH on enzyme production.

Different inoculum levels were prepared for the production of enzyme 50%, 60%, 70%, 80%, 90% v/w. The maximum enzyme production was observed at 80% v/w of inoculum fig.4.

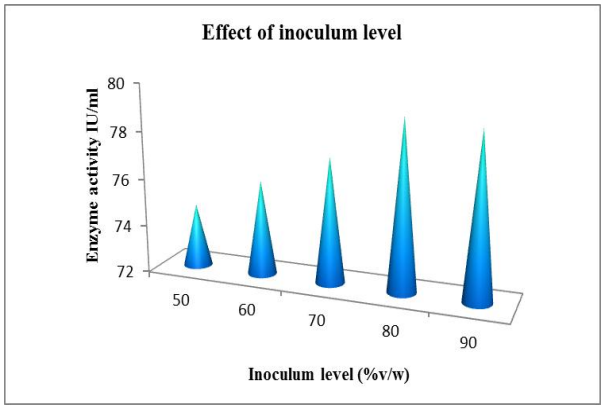

Fig.4: Effect of inoculum level on enzyme production.

Different moisture content 40%, 50%, 60%, 70%, 80%, 90%, and 100% v/w were taken in different conical flask. Moisture content is plays crucial role for the production of enzymes in SSF. High moisture content results in decreased substrate porosity, which in turn prevents oxygen penetration, this may cause bacterial contamination. The maximum activity was observed at 70% v/w of the moisture content fig.5.

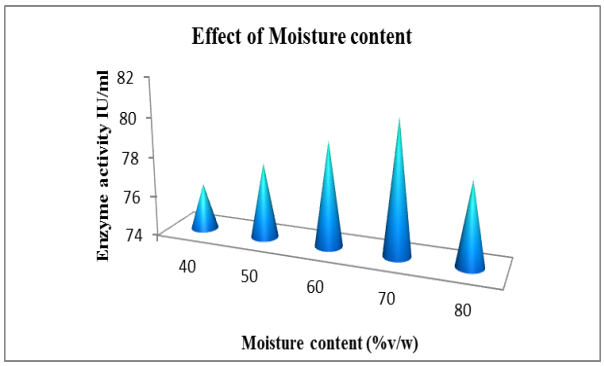

Fig.5: Effect of moisture content on enzyme production.

Five different carbon supplements were screened for the production of L-asparaginase enzyme which includes sucrose, maltose, glucose, fructose, and lactose. These are enriched with different concentrations % w/w. These results indicate that sucrose supplementation gave best improved enzyme than other supplementations fig.6.

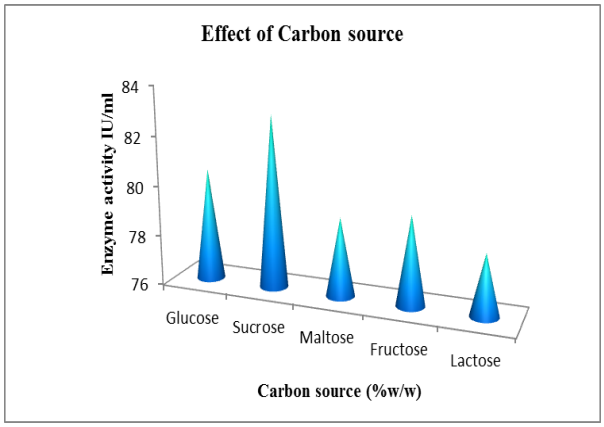

Fig.6: Effect of carbon source on enzyme production.

To determine the effect of nitrogen source on the production of enzyme, the production medium was prepared with different concentrations of L-asparagine like 0.1%, 0.2%, 0.3%, 0.4%, and 0.5% w/w were dispersed in 250ml conical flasks. These results indicate that maximum enzyme production was observed at 0.3% w/w of L-asparagine concentration fig.7.

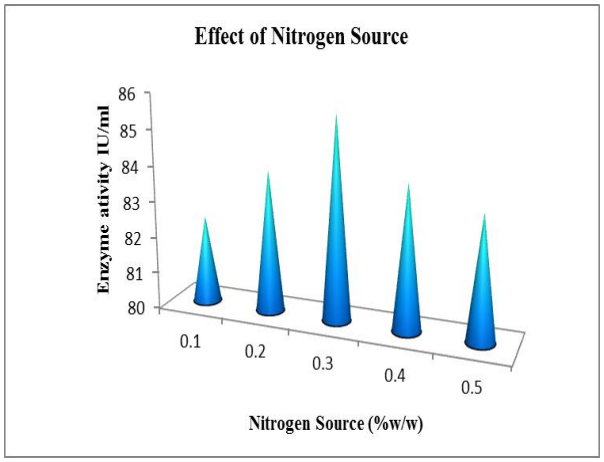

Fig.7: Effect of nitrogen source on enzyme production.

CONCLUSION

Finally we concluded that *Neolamarckia cadamba* is a promising agent for the production of enzyme which is having industrial application since it gave a significant production of L –asparaginase (85.66 U/ml) in solid state fermentation using *Streptomyces ambofaciens*. *Neolamarckia cadamba* is low cost substrate, easily available raw material and showing suitability for solid state cultivation of microbes, it was suggested as a potential substrate for L –asparaginase production in solid state fermentation.

ACKNOWLEDGEMENT

The authors express their sincere thanks to the management, Mother Teresa Pharmacy College, Sathupally, Telangana for providing the necessary facilities to carry out the research work.

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