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Production of Tumor Inhibitory *L-Asparaginase* by *Serratia marcescens*

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ABSTRACT

The present work deals with the production of *L-asparaginase* by *Artocarpus heterophyllus* as a substrate in solid-state fermentation using the microorganism *Serratia marcescens. L-asparaginase* is a chemotherapeutic agent that plays an important role in the treatment of lymphosarcoma, lymphoproliferative disorders, and acute lymphoblastic leukemia**.** *L-Asparaginase* is present in many animal tissues, bacteria, and plants, but not in mankind. *L-asparaginase* enzyme production conditions like incubation time, incubation temperature, pH, inoculum level, and moisture content were optimized. The fermentation time of 48hrs and the temperature of 38^0C, pH 6.5, inoculum level of 20%v/w, and moisture content of 70%v/w were observed optimum for the production of *L-asparaginase*. Different carbon sources were screened for their influence on enzyme activity; they are glucose, sucrose, fructose, and lactose used as supplements. Among these supplements, sucrose gave a better yield. 0.6% w/w of L-asparagine as nitrogen source was observed optimum for the production of *L-asparaginase*. The conclusion that *Artocarpus heterophyllus* is a promising agent for industrial application since it gave a significant L–asparaginase (60.24 U/g) activity in solid-state fermentation.

INTRODUCTION:

Solid-state fermentation (SSF) is defined as 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 the fermenting agent[1]. A large amount of research has been conducted upon the biosynthesis of *L-asparaginase*[2] demonstrated antitumor activity. *L-asparaginase* is produced throughout the world by submerged fermentation (SF). This 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 asparaginase has been particularly studied for its 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 pancreatic carcinoma [7] and bovine lymphomosarcoma [8].Therefore, the aim of the present 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: *Artocarpus heterophyllus* was collected from the local market and dried naturally and powdered, packed, and stored until further use.

Microorganism: *Serratia marcescens* was used for the production of *L-asparaginase* enzyme using *Artocarpus heterophyllus* as substrate. Nutrient agar medium was used for the maintenance and subculturing of the microorganism.

Preparation of Inoculum: Streaking is done from the old cultures of *Serratia marcescens* on pure agar slants of nutrient agar medium and incubates them at 30^0C for 3 days.

Development of Inoculum: 10ml of sterile distilled water was added to the cells from 3-day old slant; from that 1ml of suspension containing approximately 10^5-10^6 cells/ml were used as the inoculum to each flask.

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 the 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. The contents were mixed thoroughly 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 a rotary shaker, to extract the liquid from bacterial cells. That extract was filtered through Whatman filter paper and was centrifuged at 8000 rpm for 15 min. [9]

Enzyme Assay: *L-asparaginase* enzyme activity was determined by measuring the amount of ammonia formed by nesslerization. 0.5 mL sample of the 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 time on enzyme production, the medium incubates at different time intervals and the maximum L-asparaginase activity was observed at 48hrs. After 48hrs, it was decreased due to the depletion of nutrient materials. L-asparaginase production at different time intervals is shown in fig.1.

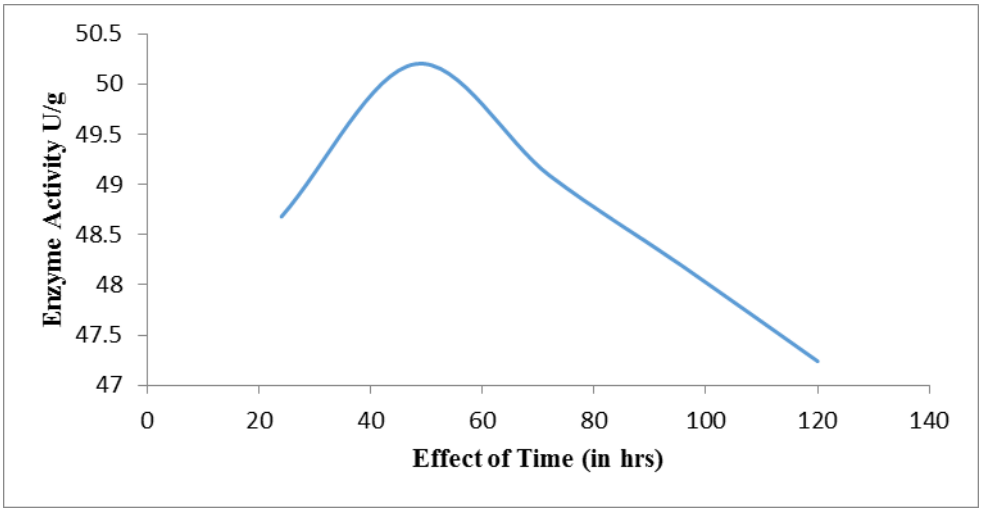

Figure No. 1: Effect of time on enzyme production

The temperature of the substrate is very critical in SSF as it ultimately affects the growth of the microorganism. The maximum amount of L-asparaginase was observed at 38^0Ctemperature Fig.2.

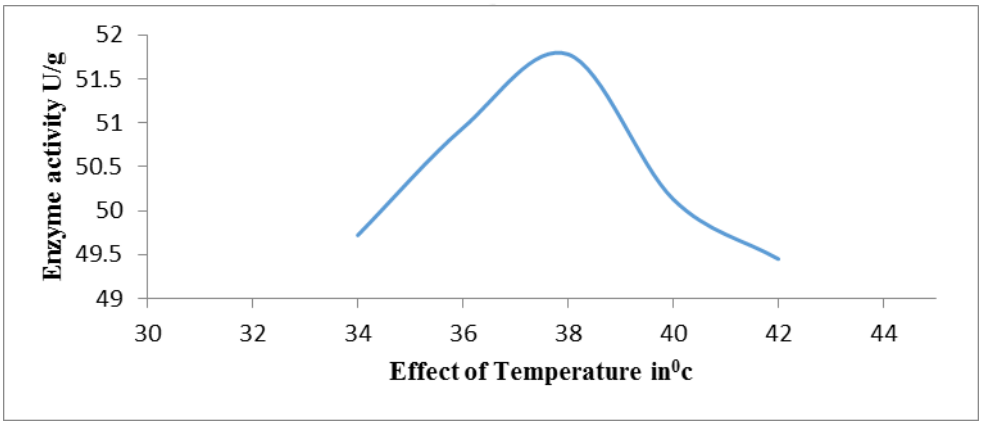

Figure No. 2: Effect of temperature on enzyme production

To determine the effect of pH, the bacterial nutrient medium was adjusted with different pH ranges 6.0, 6.5, 7.0, 7.5, and 8.0. The maximum production of L-asparaginase was recorded at pH 6.5 fig.3.

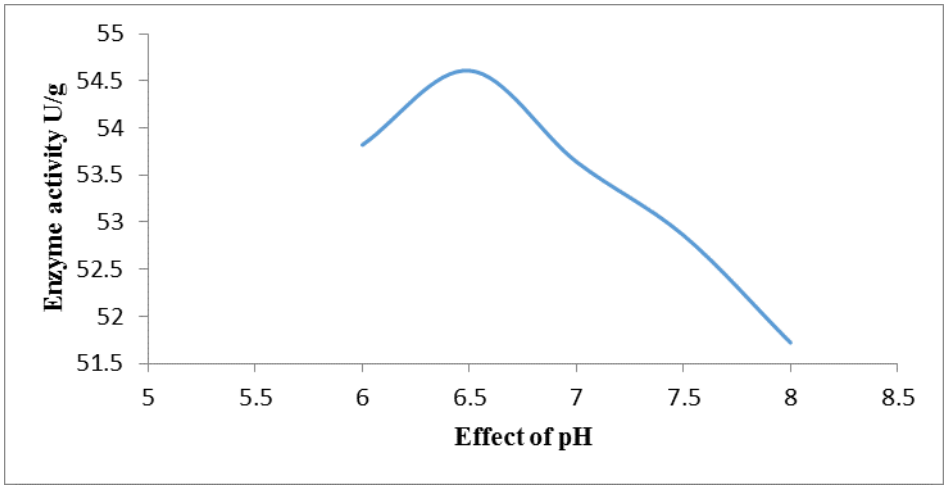

Figure No. 3: Effect of pH on enzyme production

Different inoculum levels were prepared for the production of enzyme 16%, 18%, 20%, 22%, 24% v/w. The maximum enzyme production was observed at 20% v/w of inoculum fig.4.

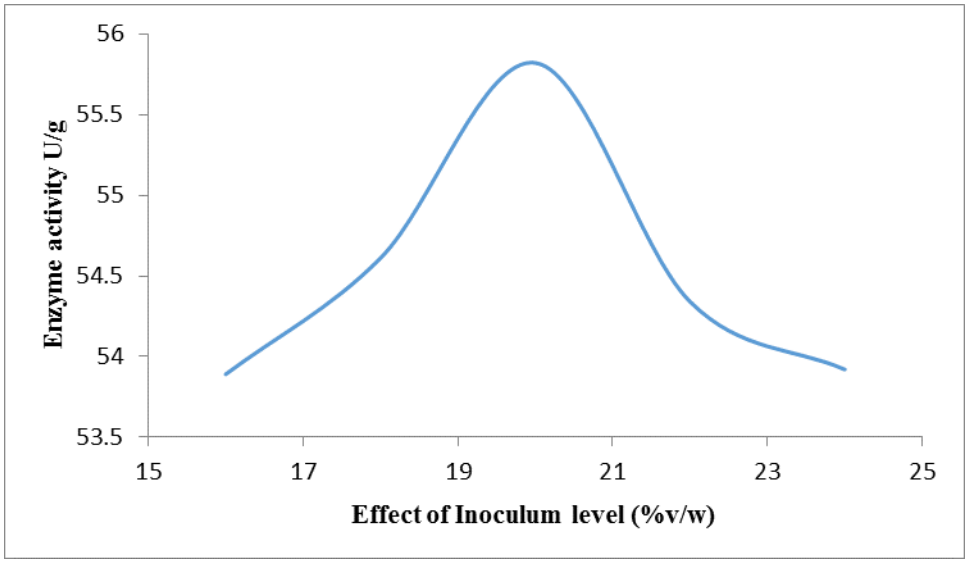

Figure No. 4: Effect of inoculums level on enzyme production

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

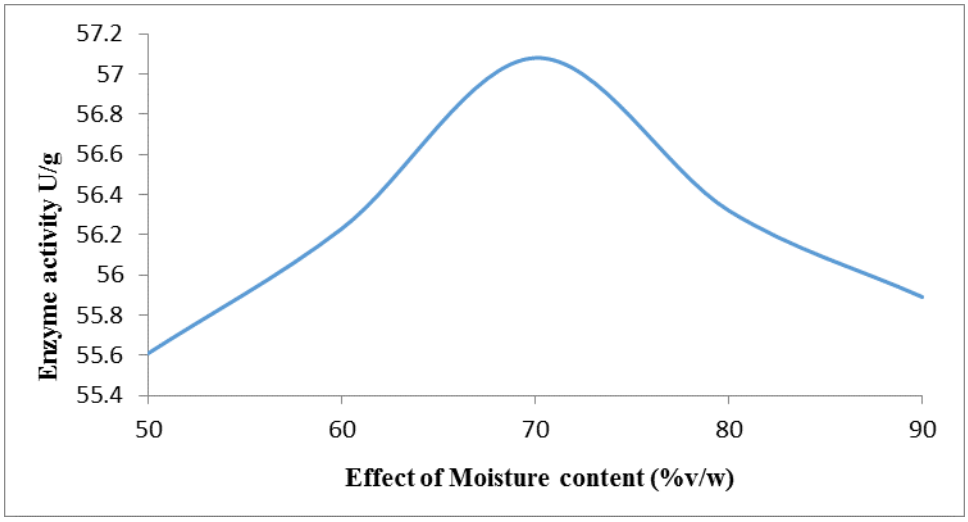

Figure No. 5: Effect of moisture content on enzyme production

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

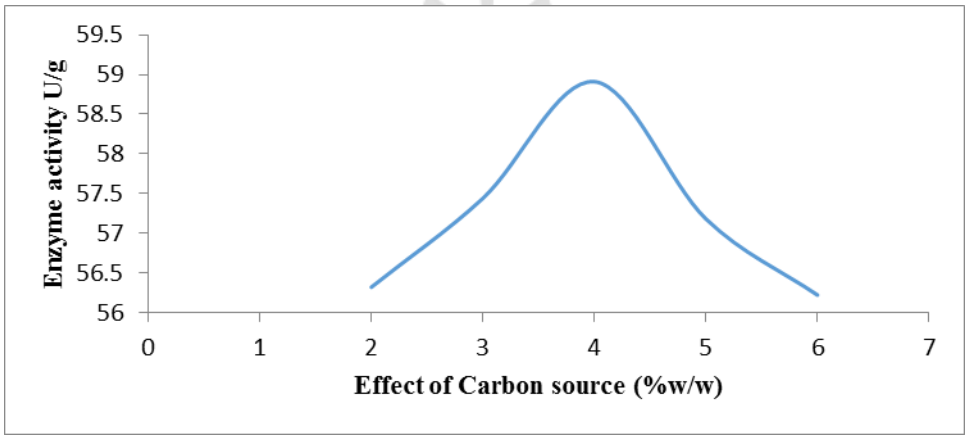

Figure No. 6: Effect of carbon source on enzyme production

To determine the effect of L-asparagine on the production of enzyme, the production medium was prepared with different concentrations of L-asparagine like 0.2%, 0.3%, 0.4%, 0.5%, 0.6% and 0.7% w/w were dispersed in 250ml conical flasks. The result indicates that maximum enzyme production was observed at 0.5% w/w of L-asparagine concentration fig.7.

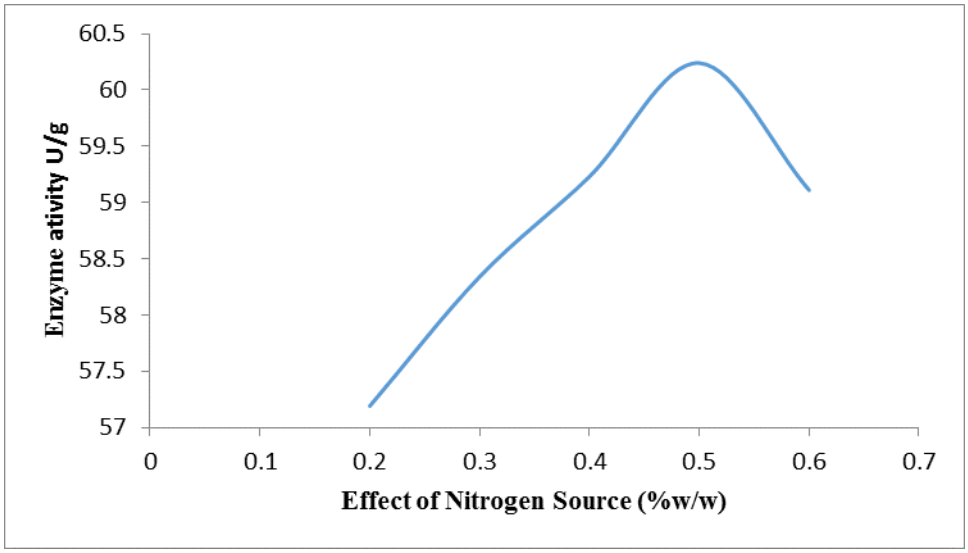

Figure No. 7: Effect of nitrogen source on enzyme production

CONCLUSION:

Finally, we concluded that *Serratia marcescens* is a promising agent for industrial application since it gave a significant *L –asparaginase* (60.24 U/g) activity in *Artocarpus heterophyllus* under solid-state fermentation. As *Artocarpus heterophyllus*is low-cost substrate, easily available, and showing suitability for solid-state cultivation of microbes, it is suggested as a potential substrate for L –asparaginase production.

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