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L-AsparaginaseProduction by Aspergillus Awamori

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ABSTRACT: The present research work discribes the production of L-asparaginase using Artocarpus heterophyllus as substrate byAspergillus awamori in a solid state fermentation.L – asparaginase is an important therapeutic enzyme used for the treatment of acute lymphoblastic leukemia, lymphosarcoma, lymphoproliferative disorders and acute lymphocytic leukemia. Normally L-Asparaginase enzyme existed in many animal tissues, plant tissues and bacterialcell, but not in human cells.In the present research, L-asparaginase enzyme production was carried out by observing differentparameters like incubation time, incubation temperature, pH, inoculum level and moisture content were noted. Different carbon supplements were checked for their influence on enzyme production; they are glucose, sucrose, maltose, fructose, and lactose. The incubation time of 48hrs, the temperature of 28^0C, pH 5.0, inoculum level of 20%v/w and moisture content of 70%v/w were observed for L-asparginase enzyme production. Among the carbonsource, glucose gave better production when compared with other carbon supplements. Nitrogen source like L-asparagine with different concentrations were checked and 0.5% w/w gave best enzyme production. Finalconclusion is that Artocarpus heterophylluscould be a promising substrate for industrial application since it produces a significant L–asparaginase (63.24IU/ml) activity in solid state fermentation.

Key words: Artocarpus heterophyllus,Aspergillus awamori, Solid-state fermentation

I. INTRODUCTION:

A major amount of researches were conducting research on the biosynthesis of L-asparaginase demonstrating the anticancer activity. L-asparaginase is an important therapeutic enzyme used for the treatment of acute lymphoblastic leukemia, lymphosarcoma, lymphoproliferative disorders and acute lymphocytic leukemia. The mechanism of action of L-asparaginase is to inhibit the growth of tumor cells[1-5]. Cells are able to produce the amino acid L-asparagine, which is important for its cell function, because presence of the enzyme asparagine synthetase. Tumor cells lack the enzyme asparagine synthetase, being not able to produce L-asparagine for their growth and development. Solid-state fermentation is aprocess that takes place on a non-soluble material thatacts both as support and a source of nutrients,with a reduced among of water, under the actionof fermenting agent[6-9]. L-asparaginase production was carried out throughout the worldbySolid-state fermentation and submerged fermentation[10-15]. Solid-state fermentation is a very effective fermentation technique that yields high production in many times when compared to submerged fermentation. Submerged fermentationtechniquehas many disadvantages, like lowconcentration production, and consequenthandling, reduction, and disposal of largevolumes of water during the downstreamprocessing. Therefore, the submerged fermentation technique is a costintensive, highly problematic, and poorlyunderstood unit operationL-Asparaginase is present in many animal tissues, bacteria and plants, but not in mankind. Microbial asparaginaseshave been particularly studied for theirapplications as therapeutic agents in the treatmentof certain types of human cancer [16-23]. L-asparaginasefrom two bacterial sources (E. coli and Erwiniacarotovora) is currently in clinical use for thetreatment of acute lymphoblastic leukemia. Itis also used for the treatment of variouscarcinoma and bovine lymphomosarcoma.Therefore, the aim of the present research work is todiscovery of a new L-asparaginase producer thatis serologically different from the previouslyreported ones, but one that has similar therapeuticeffects.

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II. MATERIAL AND METHODS:

Substrate:Artocarpus heterophyllusleaves were collected from our college garden, Sathupally and dried naturally, powdered, packed and stored until further use.

Microorganism:Aspergillus awamori was used for the production of L-asparginase enzyme using Artocarpus heterophyllusleaves as substrate. Potato dextrose agar medium was used for sub culturing and maintenance of the microorganism.

Preparation of Inoculum:Streaking was done from the old cultures of Aspergillus awamori on purepotato dextrose agar medium and incubated them at 26^0C for 3 days.

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

Solid State Fermentation:Solid state fermentation was carried out in 250-mL erlenmeyer flask 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 of the medium was adjusted to 6.0. Solid state fermentation was accomplished by taking 10g of substrate in 250ml Erlenmeyer flask, moistening it with 5ml of production medium, mixed thoroughly and placed in an autoclaved at 15lb pressure, 121^0C for 15min for sterilization. After sterilization, it wascooled;then the flasks were inoculated with 1ml of inoculumand incubated in an incubator [24-30]

Enzyme Extraction:The enzyme extraction was carried out at a temperature of 36^0C for 24hrs interval. The solid statefermentation material corresponding to one Erlenmeyer flask was mixed with 100ml of Sodium Phosphate buffer and rotated for 45mins with the help of Rotator shaker. After 45min the extraction was filtered in Whatman filter paper, from that 1ml of the extract was placed in to a centrifugal tube and centrifuged at 10000rpm for 10mins.

Enzyme Assay:L-asparaginase enzyme activity was detected by measuring the amount of ammonia formed by nesslerization. The free suspension in centrifugation tube of 0.2ml was taken into a centrifugal tube and mixer with 0.04ml L-Asparine and followed by the addition of 0.8ml of 0.1M Sodium borate. Itwas incubated for 10mins to liberate the ammonia, 0.5ml of 15% trichloroacetic acidwas added to the centrifugal tube and centrifuged for 10mins at 10000rpm. From the supernatant liquid 1.0ml was taken and mixed with1.0ml Nessler's reagent to detect liberated ammonia at 480nm in UV equipment. One unit(U) of L-asparaginase was the amount of enzyme which liberates 1µmole of ammonia in 1 min at 37^0C[31-33].

Results and Discussion:

To determine the effect of fermentationtime on enzyme production, the medium incubate at different time intervals, after completion of every 24hrs, enzyme extraction process was done and the maximum L-asparaginase activity was noted at 48hrs. After 48hrs, it was decline due to depletion of nutrients in the medium. L-asparaginase enzyme production at different time intervals was shown in the fig.1.

Determination of Enzyme Activity:

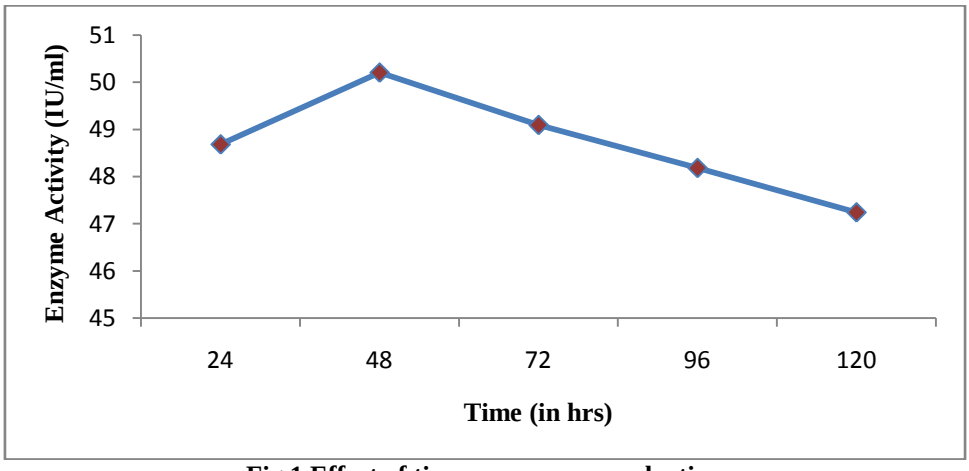

Fig.1 Effect of time on enzyme production

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The temperature wascrucial in Solid state fermentationbecause it ultimately affects the growth of the microorganism. To determine the effect of fermentation temperature on enzyme production, the medium was incubated at different temperatures, after completion of 48hrs, enzyme extraction process was done. The maximum production of L-asparaginase enzyme was noted at 28^0c temperature Fig.2.

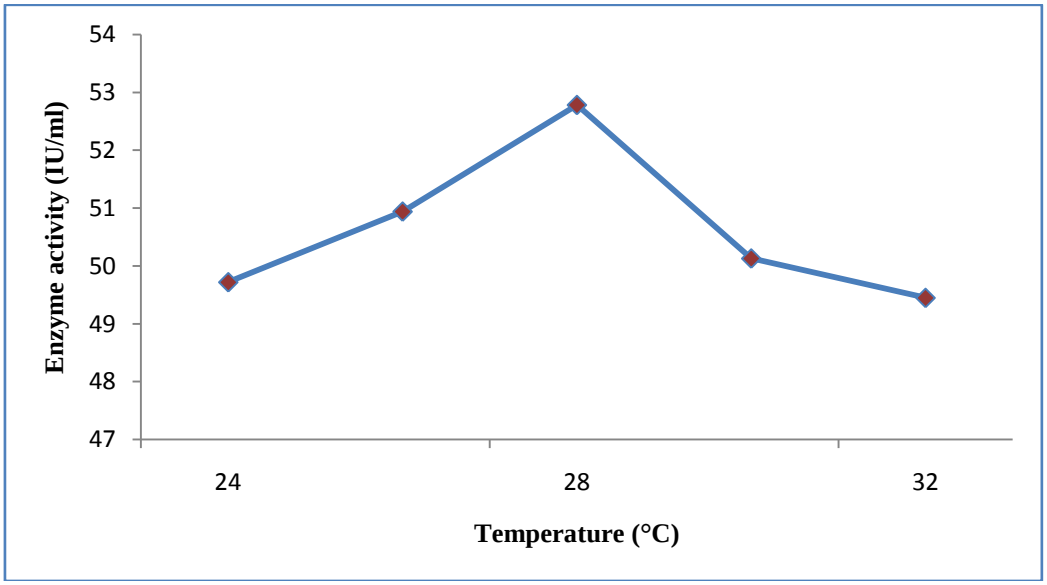

Fig.2. Effect of temperature on enzyme production

Every enzyme has an optimum pH when it was more effective. An increasing or decreasing pH reduces enzyme activity by changing the ionization. To determine the effect of pH, the nutrient medium was adjusted to different pH ranges 3, 4, 5, 6and 7. The maximum enzyme production of L-asparaginase was noted at pH 5.0fig.3.

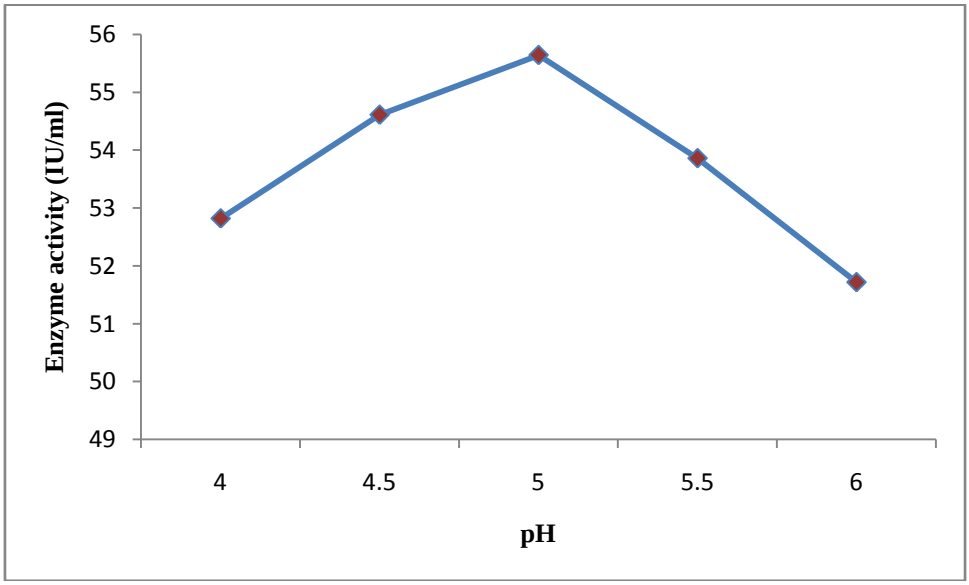

Fig.3. Effect of pH on enzyme production

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When inoculum size was increased from 5 to 10% there was increase in enzyme production but thereafter the enzyme activity was decreased, because depletion of nutrients by the enhanced biomass, which resulted diminishing in metabolic activity. To determine inoculum size, different inoculum levels were prepared for the production of enzyme 16%,18%, 20%,22% and24%v/w. The maximum enzyme production was noted at 20% v/w of inoculum fig.4.

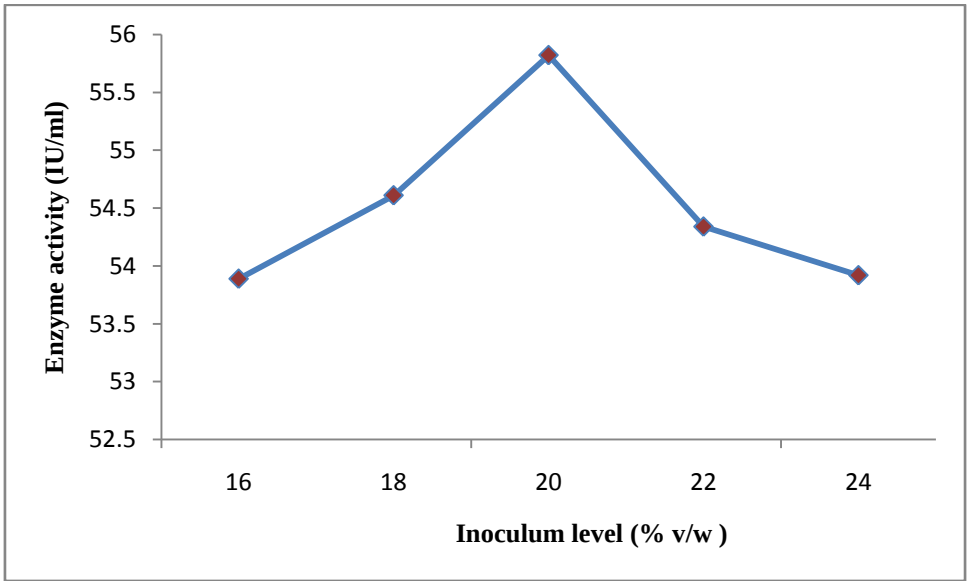

Fig.4. Effect of inoculum level on enzyme production

Moisture content in solid state fermentation is plays crucial role in the production of enzymes. High moisture content results in decreasing the substrate porosity, which may turns reduction in oxygen penetration, it may cause contamination. To determine the moisture contenton the enzyme production, various moisture content were prepared like 50%, 60%, 70%, 80% and 90%v/w were taken in different conical flask.. The maximum activity was noted at 70% v/w of the moisture content fig.5.

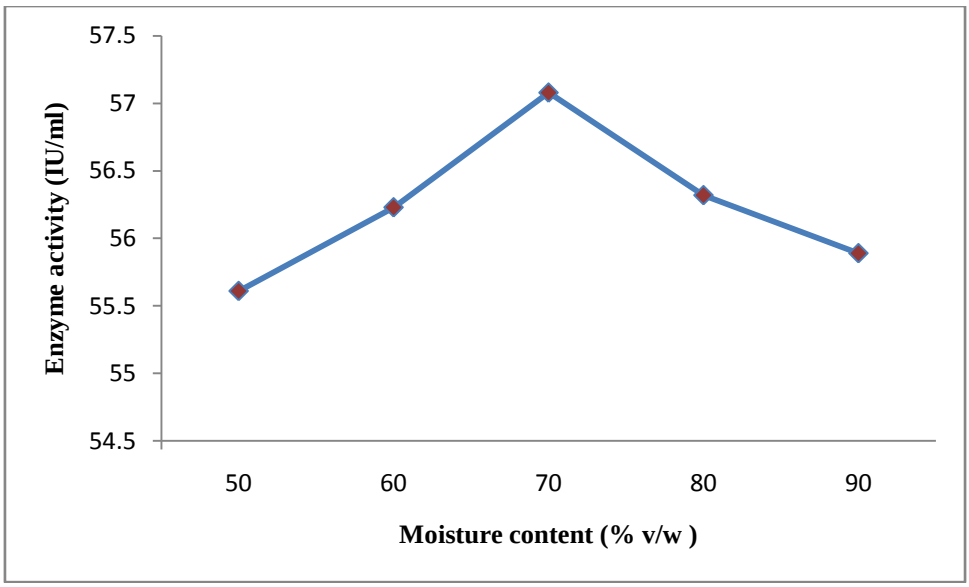

Fig.5. Effect of moisture content on enzyme production

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To determine the effect of carbon source on enzyme production, five different carbon supplements were screened for the production of L-asparaginase enzyme which issucrose, maltose, glucose, fructose, and lactose. The nutrient medium was enriched with different carbon concentrations % w/w. The result noted that glucose supplementation gave better improved enzyme production than other supplementations fig.6.

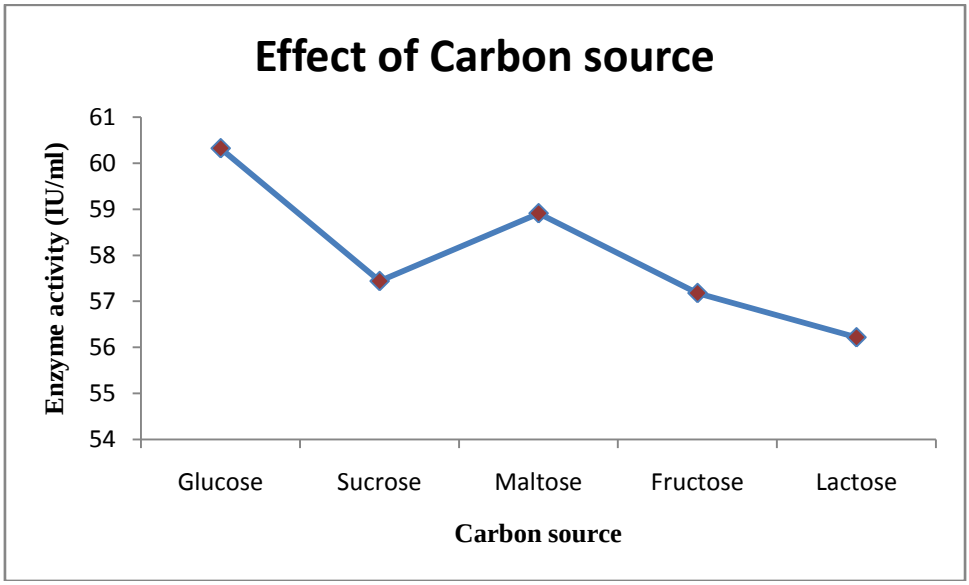

Fig.6. Effect of carbon source on enzyme production

To determine the effect of nitrogen source on the production of enzyme, the production medium was made with different concentrations of L-asparagine like 0.2%, 0.3%, 0.4%, 0.5%, 0.6% w/w were dispersed in 250ml conical flasks. The results indicate that maximum enzyme production was noted at 0.5% w/w of L-asparagine concentration fig.7.

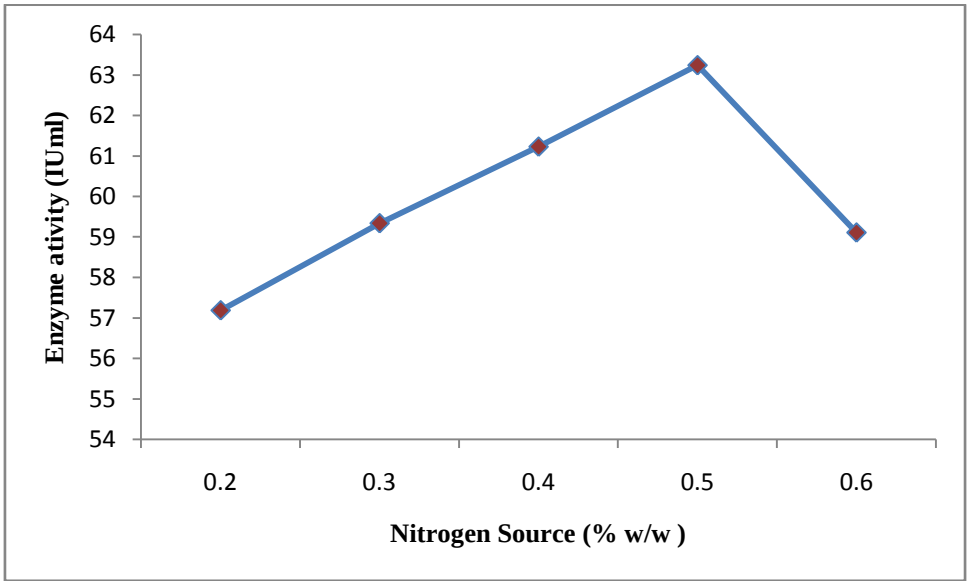

Fig.7. Effect of nitrogen source on enzyme production

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III. CONCLUSION:

Finally we concluded that Artocarpus heterophyllus is a promising agent for the production of enzyme which is having industrial application,and important therapeutic use for the treatment of acute lymphoblastic leukemia, lymphosarcoma, lymphoproliferative disorders and acute lymphocytic leukemia. It gave a significant L –asparaginase enzyme production (63.24 IU/ml) in solid state fermentation using Aspergillus awamori. Artocarpus heterophyllus islow 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.

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