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Research Paper

Production of Protease Using Streptomyces Ambofaciens

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ABSTRACT: *Proteases are complex and large group of enzymes that plays a predominant role in nutritional and regulatory activities in living cells. Proteases are very essential for the physiological functions in living organisms, which helps in the breakdown of protein molecules and food materials into amino acids, they are used for energy and plays an extreme role in the process of blood clotting, cell division. The main aim of the present research work was optimization of process parameters for the production Protease enzyme using Artocarpus heterophyllus leaves as substrate in a solid state fermentation by using microorganism Streptomyces ambofaciens. Solid-state fermentation was elucidated as an activity that occurs on a non-soluble material may acts both as support and a source of nutrients, with a reduced amount of water under the process of fermentation. For the production of protease enzyme different parameters like incubation time, incubation temperature, pH, inoculum level and moisture content and were optimized. The incubation time of 72hrs, the temperature of 34^{0}C, pH 5, inoculum level of 80%v/w and moisture content of 80%v/w were marked optimum for the production of protease. Different carbon components were screened for the enzyme production; they are glucose, fructose, maltose, sucrose and lactose used as carbon supplements. Among the carbon source, glucose gave best production when compared with other carbon supplements. To determine nitrogen effect on enzyme production, potassium nitrate was taken and 0.3% w/w was observed optimum for the enzyme production. Final conclusion was that Artocarpus heterophyllus could be a promising substrate for industrial application since it produces a significant L–asparaginase (88.25 IU/ml) activity in solid state fermentation.*

KEY WORDS: *Artocarpus heterophyllus, Streptomyces ambofaciens, Solid-state fermentation.*

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I. INTRODUCTION:

Proteases are very essential for the physiological functions in living organisms, which helps in the breakdown of protein molecules and food materials into amino acids, they are used for energy and plays an extreme role in the process of blood clotting, cell division. Proteases are complex and large group of enzymes that plays a predominant role in nutritional and regulatory activities in living cells. Protease enzymes were distinguishing a wide diversity of sources such as plants, animals and microorganisms, they are mainly produced by numerous bacterial and fungal species[1- 6]. Protease enzymes are the most crucial and essential industrial enzymes that producing about 60% of the total enzymes market in the world [7, 8]. Microorganisms like bacteria and fungi produce more varieties of proteolytic enzymes. A proteolytic enzyme splits proteins in the body, which may helps in digestion or with the breakdown of proteins involved in swelling and pain. Solid-state fermentation was elucidated as an activity that occurs on a non-soluble material may acts both as support and a source of nutrients, with a reduced amount of water under the process of fermentation. Solid-state fermentation having more advantages compared with submerged fermentation because of superior volumetric productivity, use of simpler machinery, use of inexpensive substrates, simpler downstream processing, and lower energy requirements [9-14]. Microbial proteases are produced in extracellular and might be secreted in the fermentation medium. Protease enzymes have various applications in food industries, meat processing, cheese making, detergents, leather, silver recovery from photographic film, production of digestive and also used in the treatments of inflammation and virulent wounds. Microorganisms can potentially grow under various environmental conditions such as time, temperature, pH and moisture content etc, utilizing a wide variety of substrates as nutrients [15, 16]. The environmental conditions of the fermentation medium play a crucial role in the growth and development of microorganism. Nutrients can effect growth and development of the microorganisms either directly by acting on the cell surfaces or indirectly by affecting the availability of nutrients present in the medium [17]. The main aim of the present research work was optimization of process

parameters for the production Protease enzyme using *Artocarpus heterophyllus* leaves as substrate in a solid state fermentation by using microorganism *Streptomyces ambofaciens.*

II. MATERIAL AND METHODS:

Substrate: *Artocarpus heterophyllus* leaves were collected from our college ground, Sathupally, Telangana and they were naturally dried and powdered, packed and stored until further use.

Microorganism: *Streptomyces ambofaciens* was used for the optimization of process parameters for the production of Protease enzyme using *Artocarpus heterophyllus* leaves as substrate. Nutrient agar medium was used for the maintenance and sub culturing of the microorganism.

Preparation of Inoculum: Streaking was done on pure nutrient agar slants from the old cultures of *Streptomyces ambofaciens* and incubated them at 34^0C for 3 days.

Development of Inoculum: 10ml of sterile distilled water were added to the 3 days old cultured slants, from that 1ml of suspension was used as the inoculum and placed into each flask containing approximately 10^5-10^6 spores/ml.

Fermentation condition: Solid-state fermentation was carried out in 250ml conical flask containing 10g of substrate with 10ml of production medium (g/l) containing glucose 10g/l; peptone 5g/l; yeast 5g/l; $MgSO_4$ 2g/l; Na_2CO_3 10g/l; NaCl 5g/l; K_2HPO_4 1g/l; $FeSO_4$ 1g/l. Inoculum was placed to the production medium and incubated with continuous shaking. Shaker fermentation was accomplished at 36^0c with controlled agitation at 150-200 rpm. At the end of the fermentation period, the whole culture broth was centrifuged at 1000rpm for 1 hour to remove debris; the supernatant was collected and used for further experiments [18-20].

Determination of Enzyme Activity:

Enzyme Extraction :- At the end of fermentation, the enzyme produced in the culture was extracted with 100ml of distilled water by stirring for 45min using magnetic stirrer. The harvested culture was filtered through Whatmann no.1 filter paper and centrifuged at 10,000rpm for 10min. was added to the cell free culture filtrate to precipitate the protein. 0.5mL enzyme extraction was added to 3.0mL (0.6% w/v) casein solution prepared in 20mM Borax-NaOH buffer, (pH 10) and allowed to process for 10 min at 36°C. 3.2mL trichloroacetic acid solution was added stopped the reaction. The reaction mixture was kept a side for 10 min, the absorbance was measured at 280nm and related to the protease activity.

III. RESULTS AND DISCUSSION:

Protease enzymes have various applications in food industries, meat processing, cheese making, detergents, leather, silver recovery from photographic film, production of digestive and also used in the treatments of inflammation and virulent wounds. Microbial proteases are degenerating enzymes that catalyze the total hydrolysis of proteins. Proteases enzyme have various medical and pharmaceutical applications. To determine the effect of incubation time on protease enzyme production, the medium incubate at different time intervals and the maximum protease activity was optimized at 72hrs. After 72hrs, due to depletion of nutrient materials enzyme production was decreased. Protease production at various time intervals was shown in the fig.1.

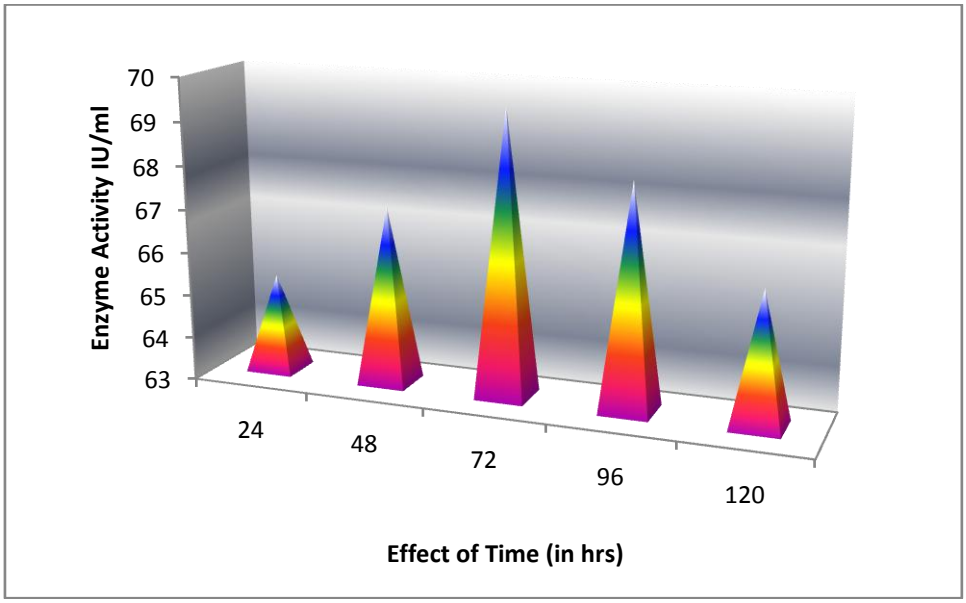

Fig.1 Effect of time on enzyme production

The temperature was an important and very critical for the production of enzyme in solid state fermentation as it ultimately influence the growth of the microorganism. The maximum yield of protease was observed at 34^0c temperature Fig.2.

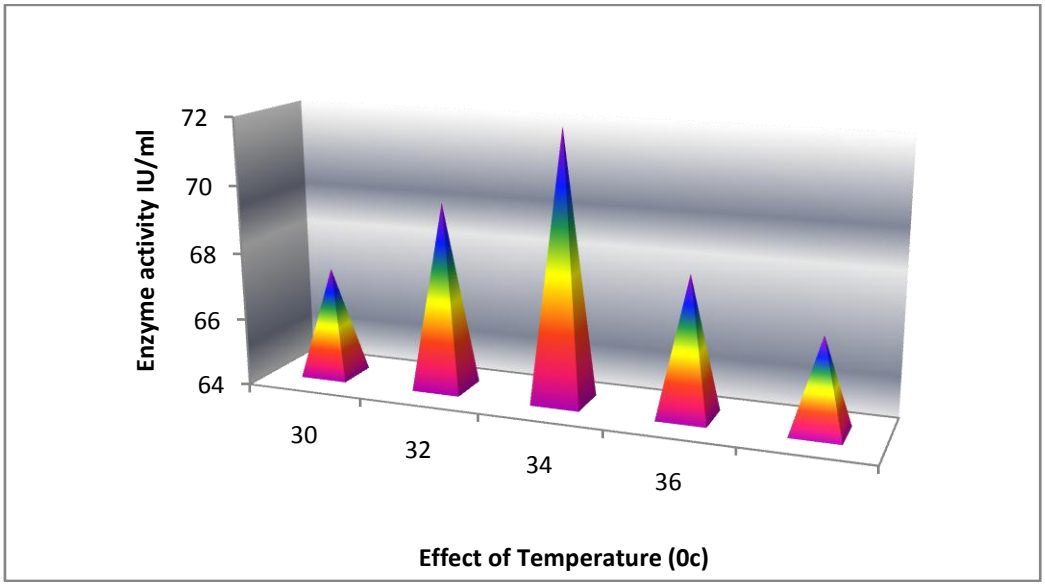

Fig.2. Effect of temperature on enzyme production

Every enzyme has an optimum pH, an increasing or decreasing pH reduces enzyme activity by changing the ionization. To optimize the effect of pH on enzyme production, the nutrient medium was prepared with different pH ranges 3, 4, 5, 6 and 7. The maximum production of protease enzyme was noted at pH 5 fig.3.

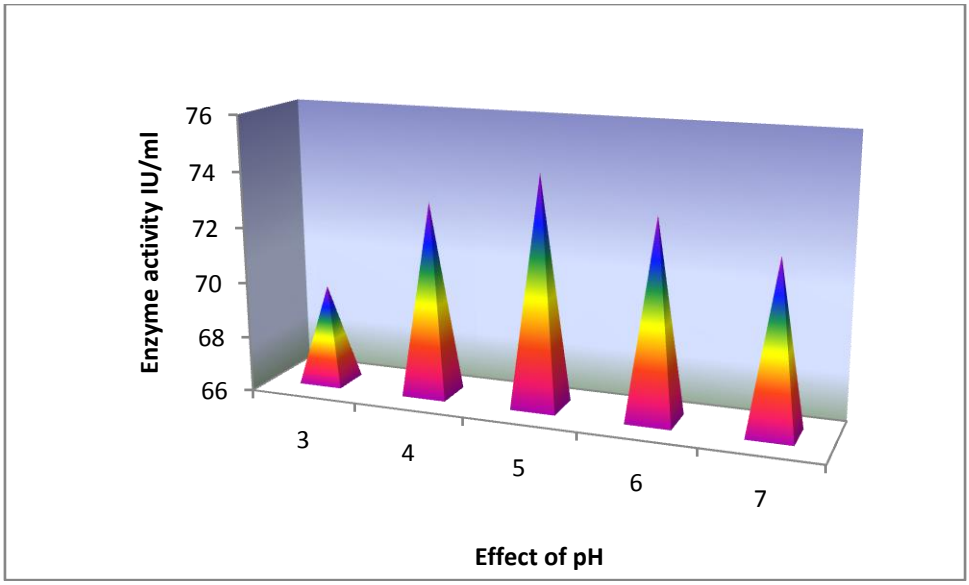

Fig.3. Effect of pH on enzyme production

When the 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 increase biomass, which resulted diminishing in metabolic activity. Different inoculum levels were prepared for the production of protease enzyme 40%, 50%, 60%, 70%, 80% 90% and 100% v/w. The maximum enzyme production was observed at 80% v/w of inoculum fig.4.

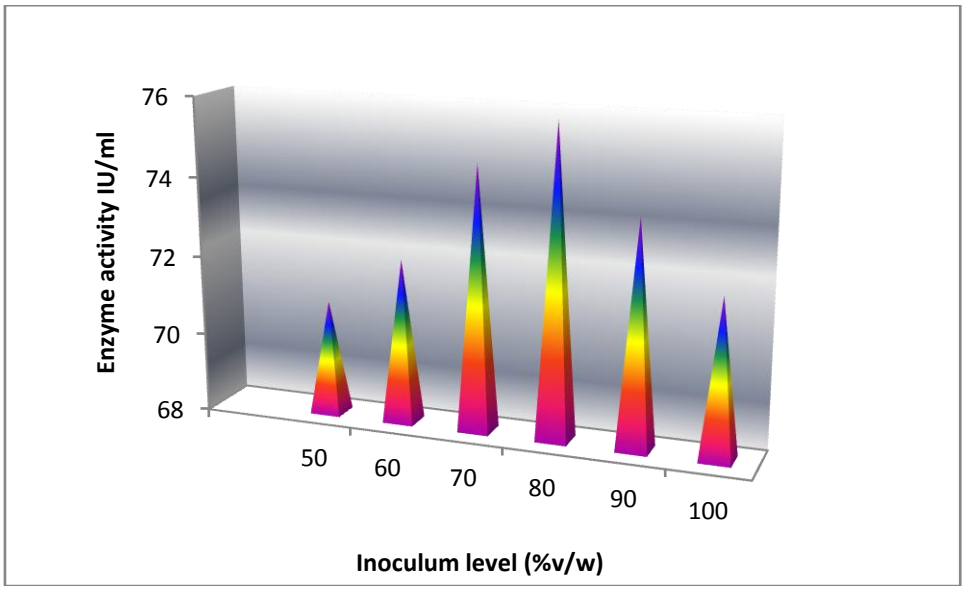

Fig.4. Effect of inoculum level on enzyme production

Moisture content plays an important role in solid state fermentation for the production of enzymes. High moisture content observes decreased substrate porosity, which prevents oxygen penetration that causes bacterial contamination. Different moisture content was optimized with 40%, 50%, 60%, 70%, 80%, 90%, and 100% v/w. The maximum enzyme activity was optimized at 80% v/w of the moisture content fig.5.

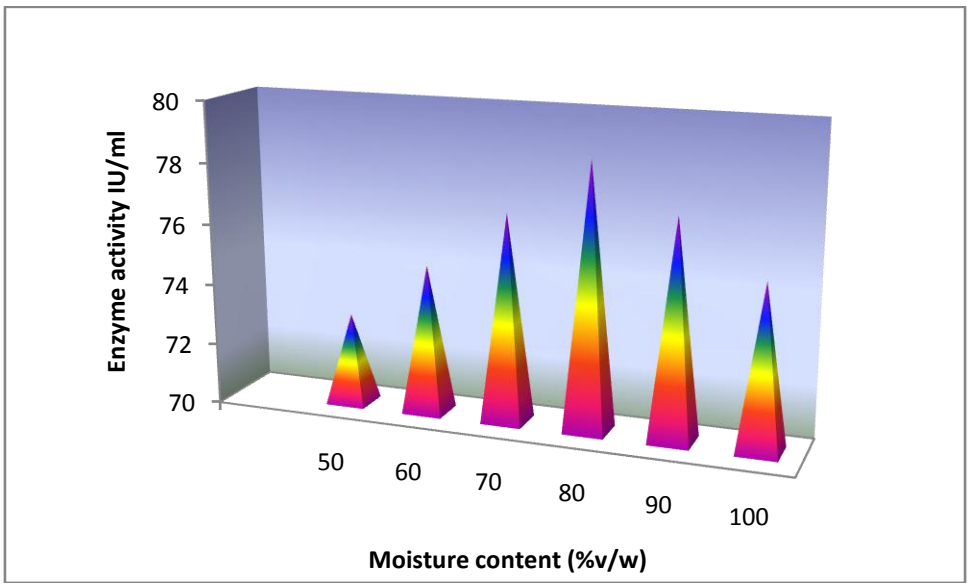

Fig.5. Effect of moisture content on enzyme production

Different carbon components were screened for the enzyme production; they are glucose, fructose, maltose, sucrose and lactose used as carbon supplements. Among the carbon source, glucose gave best production when compared with other carbon supplements. Production medium was prepared with different concentrations of glucose like 1, 2, 3, 4, 5 and 6 %w/w. The result shows that maximum enzyme production was observed at 3% w/w of glucose concentration fig.6.

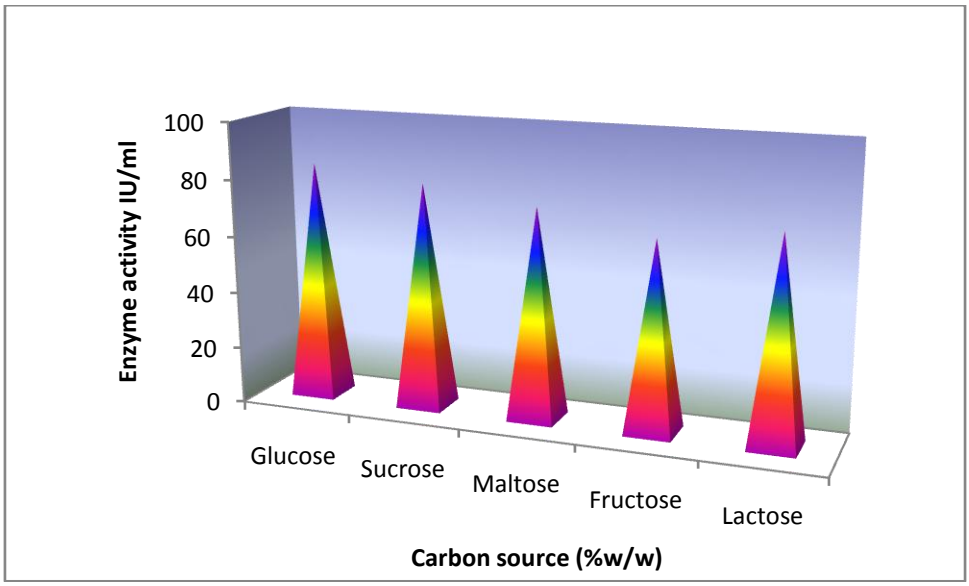

Fig.6. Effect of carbon source on enzyme production

To determine the effect of nitrogen on protease enzyme production, the production medium was made with different concentrations of potassium nitrate like 0.1%, 0.2%, 0.3%, 0.4%, and 0.5% w/w were optimized. The results indicate that maximum production of enzyme was recorded at 0.3% w/w concentration fig.7.

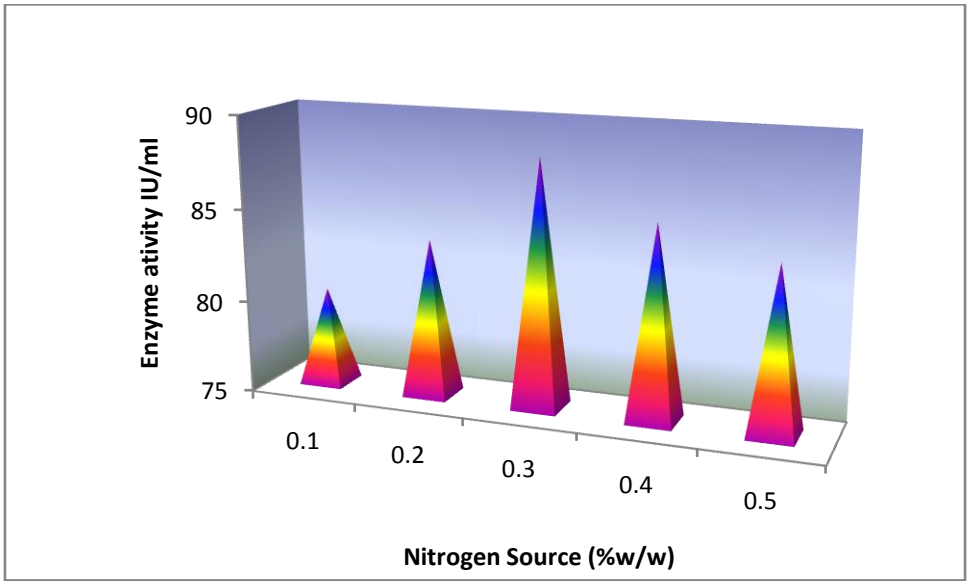

Fig.7. Effect of nitrogen source on enzyme production

IV. CONCLUSION:

Finally we concluded that *Artocarpus heterophyllus* leaves were promising agent that produce significant protease enzyme (88.25 IU/ml) by *Streptomyces ambofaciens* under solid state fermentation. Protease enzymes were distinguishing a wide diversity of sources such as plants, animals and microorganisms, they are mainly produced by numerous bacterial and fungal species. As *Artocarpus heterophyllus* leaves were easily available raw material and showing suitability for solid state cultivation of microorganisms, the lab-scale study on protease production from *Artocarpus heterophyllus* leaves as major substrate might give the basic information of further development for large scale protease production.

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