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EFFECT OF ORGANIC NITROGEN SOURCE FOR PRODUCTION OF FIBRINOLYTIC ENZYME AS A "CLOT BUSTING DRUG BY *ASPERGILLUS JAPONICUM*

Saroj Yadav*[1] and Siddalingeshwara K. G.[2]

[1]Research and Development Centre, Bharathiar University. Coimbatore.
[2]Scientific & Industrial Research Centre, Bangalore.

***Corresponding Author: Saroj Yadav**

Research and Development Centre, Bharathiar University. Coimbatore.

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ABSTRACT

Cardiovascular disease (CVDs) is caused by disorders of the heart and blood vessels, and includes coronary heart disease (heart attacks), cerebrovascular disease (stroke), raised blood pressure (hypertension), peripheral artery disease, rheumatic heart disease, congenital heart disease and heart failure. Fibrinolytic enzymes are agents that dissolve fibrin clots extracted from microbes were considered to be a potential thrombolytic agent for the treatment of clot-dissolving in the cases of Myocardial infarction. The fungal strains i.e *Aspergillus japonicum* KGSY 05 were isolated from different soils from different regions of Karnataka and screened for fibrinolytic activity by plate assay. The potential strain *Aspergillus japonicum* KGSY 05 were used for the enhance the biosynthesis of fibrinolytic enzyme by supplementation of nitrogen (organic) sources in range of 0.25% to 1.25%. The different organic nitrogen sources such as yeast extract, beef extract and peptone were employed. The peptone showed better yield of 123 IU fibrinolytic enzyme production and yeast extract (1.0%) 68 IU, beef extract (1.0%) 103 IU.

KEYWORDS: Clot buster drug, fibrin carbon source, *Aspergillus tamari.*

INTRODUCTION

Enzymes as drugs have two important features that distinguish them from all other types of drugs. First, enzymes often bind and act on their targets with great affinity and specificity. Second, enzymes are catalytic and convert multiple target molecules to the desired products. These two features make enzymes specific and potent drugs that can accomplish therapeutic biochemistry in the body that small molecule cannot. These characteristics have resulted in the development of many enzyme drugs for a wide range of disorders.

Thrombosis, that is the formation or presence of a thrombus (a clot of coagulated blood attached at the site of its formation), in a blood vessel is one of the most widely occurring diseases in modern life, which often causes disturbance in haemostasis a leading cause of cardiovascular diseases causing disability and death. According to world health organization (WHO), 17 million people die of such cardiovascular diseases (CVDs) every year.[1]

Consequnces like imbalance in the haemostasis i.e. balance between coagulation and anticoagulation, may result in excessive bleeding or formation of a thrombus that can adhere to the unbroken wall of the blood vessels. Fibrin can accumulate in the blood vessels which can interfere with blood flow and lead to myocardial infarction and other serious cardiovascular diseases. Unless the blockage is removed promptly, the tissue that is normally supplied with oxygen by the vessel will die or be severly demaged.[2]

Fibrinolytic enzymes have been purified, cloned and studied from many plants, animals and microbial sources.[3] The agents are of interest as useful tools for understanding fibrinolytic mechanism and as potential therapeutic drugs.

Due to the importance of fibrinolytic enzymes production, we made an effort to synthesis of fibrinolytic enzyme from *Aspergillus japonicum* KGS Y 05 through submerged fermentation, achieved an enhanced level production of fibrinolytic enzyme by supplementation nitrogen source were carried out.

MATERIALS AND METHODS

Microorganism

The *Aspergillus japonicum* KGSY 05 strains were isolated from different soils. Soils are taken from different regions from in and around Bangalore and tentatively identified in the laboratory and confirmed by molecular level identification.

Screening of Fibrinolytic Enzyme Producers by Plate Assay

Aspergillus japonicum KGSY 05 were used to screen by fibrin plate assay (4). A mixture consisting of 2 ml of

fresh, healthy human plasma and 3 ml of 1.2% molten agarose (450C) in 10 mM Tris-HCl buffer containing 70 mM (NH4)2 SO4, 90 mM NaCl, 0.70 mM MgCl2 and 200 µl of 0.2 M CaCl2 was poured into sterile 60 mm petridish and allowed to stand for 2 h at room temperature (25 to 27^{0}C). 10 ml of Sabouraud dextrose broth was inoculated with the given fungal strain and incubated at 300C for 2 days. This culture was used for fibrin clot assay. 20µl of fungal culture containing mycelia was placed at the center of the gel matrix of the fibrin plate and incubated for 24 h at 300C. The diameters of the clear zones (plaque) were noted.

Influence of Organic Nitrogen Source for the Biosynthesis of Fibrinolytic Enzyme

A set of conical flasks with 100 ml of production medium supplemented with a particular carbon source with concentrations ranging from 0.25% to 1.25% with increments of 0.25%. The different organic nitrogen sources such as yeast extract, beef extract and peptone were used under the present study. The production medium consists (mg/100 ml) of Sucrose 3, di potassium hydrogen phosphate 0.1, MgSO4, 0.05g, KCl 0.05g, NaCl, 0.01%, FeSO4 and devoid of sucrose. The condition of the fermentation medium is as fallows. pH,6 temperature 35^{0}C and inoculums size is of 1.25ml.

Extraction of Fibrinolytic Enzymes

The samples were withdrawn periodically at 24 hrs in aseptic condition. The extract was filtered through Whatman filter No.1. The clear extract was centrifuged at 2000-3000 rpm for 15 min, supernatant were used as enzyme preparation. Thus prepared crude enzyme was used for assay of fibrinolytic enzyme.

Enzyme Assay

This was basically measured by the modified method of Anson[5], but with a few modifications. The reaction mixture contained 1 ml of 1.2% of bovine fibrin solution in Tris-HCl buffer (pH 8.0) and 1 ml of cell-free supernatant (CFS). The reaction mixture was incubated for 2 h at 37°C. Then the reaction was stopped by the addition of 2 ml of 10% (w/v) trichloroacetic acid. This was followed by centrifugation and assaying the solubilized proteins for tyrosine in the supernatant by measuring the absorbance at 750 nm.[6]

UNIT

One unit of fibrinolytic activity (U) was defined as the amount of enzyme required to liberate 1 µg of L-tyrosine/ ml/min at 37°C

RESULTS AND DISCUSSION

Aspergillus japonicum strain were isolated and screened for fibrinolytic enzyme production by plate assay and the strain were confirmed by molecular level identification. The potential strain were labeled as *Aspergillus japonicum* KGSY 05.

The organic nitrogen sources greatly influence the growth of any organism. In the present study various organic nitrogen sources like yeast extract, malt extract, peptone and urea were supplemented at 0.25%, 0.50%, 0.75%, 1% and 1.25% levels to the synthetic medium for the production of fibrinolytic enzyme by *Aspergillus japonicum* KGSY 05. The results [Fig. 1 Fig. 2 and Fig. 3] revealed that the production of fibrinolytic enzyme increased with the increase in the organic nitrogen concentration upto 1.0%, thereafter no significant increase in fibrinolytic enzyme was noticed on all the days of fermentation with all nitrogen sources.

Thus, nitrogen sources like yeast extract (1.0%) 68 IU, beef extract (1.0%) 103 IU and peptone (1.0%) 123 IU influenced the fibrinolytic enzyme production respectively by *Aspergillus japonicum* KGSY 05 strain. Amongst various organic nitrogen sources tested, peptone produced maximum 123 IU amount of fibrinolytic enzyme and emerged as best organic nitrogen source for the enhancement of fibrinolytic enzyme production by employing strain *Aspergillus japonicum* KGSY 05 under submerged fermentation.

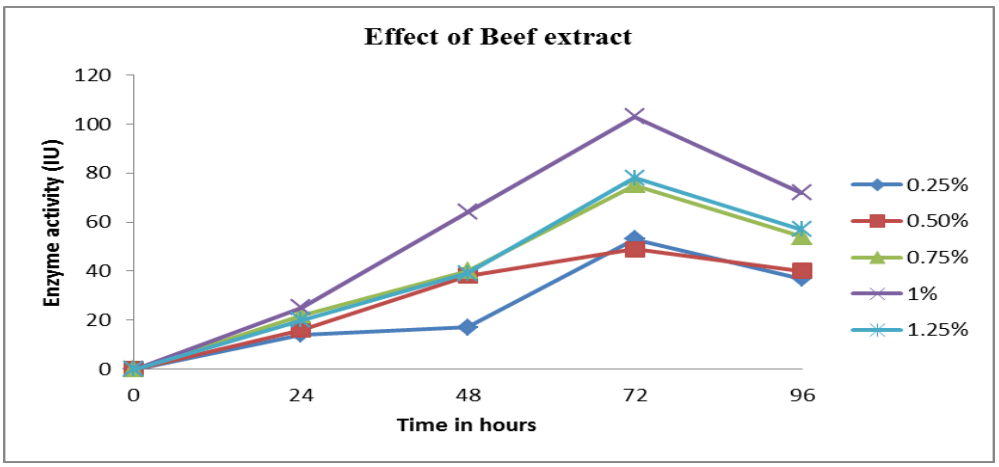

Fig. 1: Effect of Yeast extract on Fibrinolytic enzyme production.

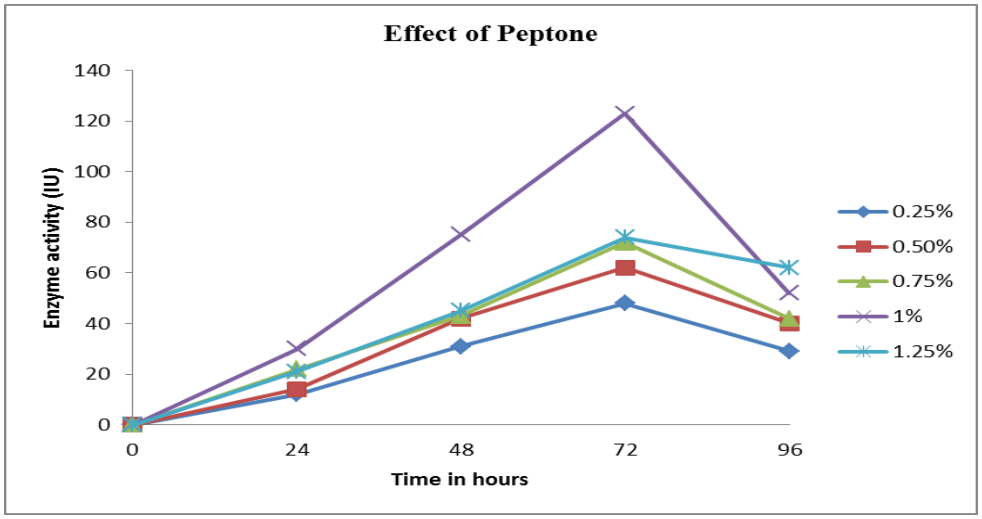

Fig. 2: Effect of Beef extract on Fibrinolytic enzyme production.

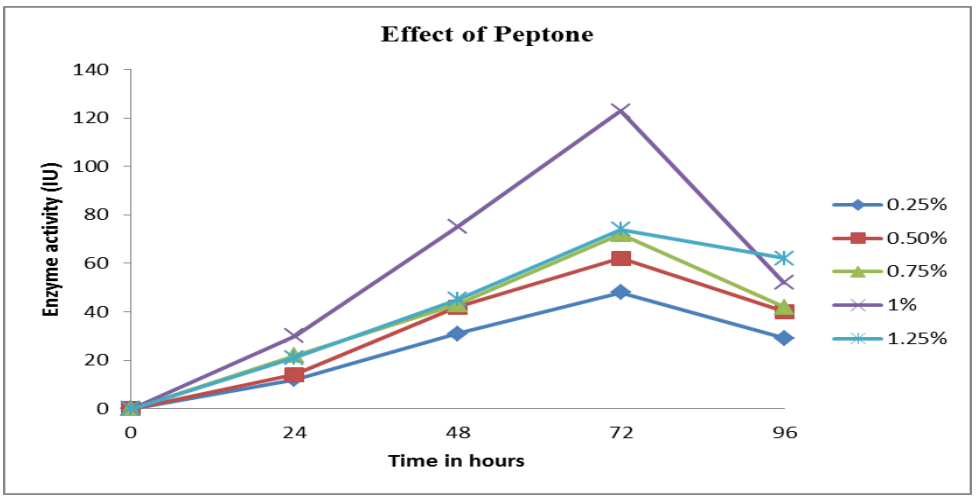

Fig. 3: Effect of Peptone on Fibrinolytic enzyme production.

Essam *et al.* 2012.,[7] were reported that nitrogen sources were examined (casein soya peptone) of all the nitrogen sources tested, soy peptone was found to be the most promising one and the corresponding fibrinolytic activity is 50 unit/m. Very poor enzyme activities were obtained. While much higher activities were obtained by using the organic nitrogen sources. Casein gave less enzyme activity in compare with the other organic nitrogen sources. Liu *et al.* 2005.,[8] who show that soy peptone was found to be the best one, for fibrinolytic activity. Maheshkumar *et al.* 2014.,[6] were studied nitrogen sources for fibrinolytic enzyme production *Bacillus subtilis* RJAS19 and Chitte and Dey, 2002[9] were discussed production of a fibrinolytic enzyme by thermophilic *Streptomyces* species by using nitrogen sources.

Ponnuswamy Vijayaraghavan, 2017.,[10] were screened different nitrogen sources, 1% casein, peptone, beef extract, yeast extract, urea and gelatin were supplemented with cow dung substrate. The lowest fibrinolytic activity was registered with urea (435±3.87 U/g) and the highest with peptone (1127±1.38 U/g).

CONCLUSION

The present investigation highlights on production of fibrinolytic enzyme from *Aspergillus japonicum* KGSY 05 through submerged fermentation. The isolation and screening were carried out. The process economization were carried out by supplementation of nitrogen source for enhanced level of fibrinolytic enzyme and it is achieved by supplementation of peptone at 1% level to the fermenting medium.

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