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RESEARCH ARTICLE

SCREENING AND BIOSYNTHESIS OF FIBRINOLYTIC ENZYME FROM *ASPERGILLUS JAPONICUM*

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

[1]Research and Development Centre, Bharathiar University, Coimbatore, India

[2]Scientific & Industrial Research Centre, Bangalore, India

**Corresponding Author's E-mail: siddha_lingeshwar@rediffmail.com*

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ABSTRACT

Fibrin is a protein that forms in the blood clots after trauma or injury. This is essential to stop blood loss. There are more than twenty enzymes in the body that assist in clotting of the blood, while only one that can break the clot down. It is an endogenously produced fibrinolytic enzyme called plasmin. Streptokinase is an extracellular metallo-enzyme produced by beta-haemolytic *streptococcus* and is used as an effective and cheap clot-dissolving medication in some cases of myocardial infarction (heart attack) and pulmonary embolism. It belongs to a group of medications known as fibrinolytics. Fibrinolytic enzymes can be found in a variety of foods, such as Japanese Natto, Tofuyo, Korean Chungkook-Jang soy sauce, and edible honey mushroom. Fibrinolytic enzymes producing *Aspergillus japonicum* KSS 05 strain were screened for the production by fibrin plate assay method. The maximum zone of fibrin hydrolysis were found 6 mm diameter. Further the *Aspergillus japonicum* KSS 05 were employed for the production by submerged fermentation and it showed 235 IU by pH 6, temperature 30^0C and 1 ml inoculums size.

Key words: Fermentation kinetics, effect of pH, fibrinolytic enzymes and fibrin

INTRODUCTION:

Fibrinolytic enzyme is well known as a sub class of protease, which has an ability to degrade fibrin [1-3]. Blood clots (fibrin) are formed from fibrinogen by thrombin (EC 3.4.21.5) and are lysed by plasmin (EC 3.4.21.7), which is activated from plasminogen by tissue plasminogen activator [4].

Accumulation of fibrin in the blood vessels usually results in thrombosis, leading to myocardial infarction and other cardiovascular diseases [5]. These diseases are the leading causes of death throughout the world [6]. Thrombolytic agents convert plasminogen to plasmin, lyse the clot by breaking down the fibrin contained in a clot.

Currently fibrinolytic enzymes that dissolve blood clots and show promise for thrombosis therapy have been successfully identified from various sources. A wide range of microorganisms has been screened for their fibrinolytic properties [7]. Fibrinolytic enzymes have been reported from various bacterial species of *Bacillus* [8], *Staphylococcus* [9], *Coryneform bacteria* [10], *Pseudomonas* [11] and *Alteromonas* [12]. Some fungi have also been found to have high fibrinolytic activity, as *Aspergillus ochraceus* 513 [13], *Fusarium* sp.[14], *Rhizopus chinensis* 12 [15] and *Penicillium* sp.[16]. In addition, fibrinolytic enzymes produced from different species of mushrooms [17-19].

Our aim of the present study is to screen and produce the fibrinolytic enzymes through submerged fermentation by optimizing fermentation parameters from *Aspergillus japonicum*.

MATERIALS AND METHODS:

Experimental microorganism

Aspergillus japonicum were isolated from soils collected from different regions in and around Bangalore. The *Aspergillus japonicum* were isolated by using Czapek Dox's media and tentatively identified in the laboratory and confirmed at Agharakar Research Institute, Pune.

Screening of fibrinolytic enzyme by fibrin plate assay

Fibrinolytic activity was determined using the method described by Astrup and Mullertz [20]. The fibrin agarose plate was made to a 1 mm thickness, and contained agarose (1.2% w/v), bovine fibrinogen (0.4% w/v), and bovine thrombin (20 U/mL) in a petridisc, and the clot was allowed to stand for 1 h at room temperature. Then, 10 µL of sample enzyme solution was carefully placed onto the plate. The plate was incubated for 5 h at 37ºC and the diameter of the lytic zone was measured and the clear transparent region was observed in which fibrin is

hydrolyzed (Plate-1). This diameter is directly proportional to the strength of the fibrinolytic activity.

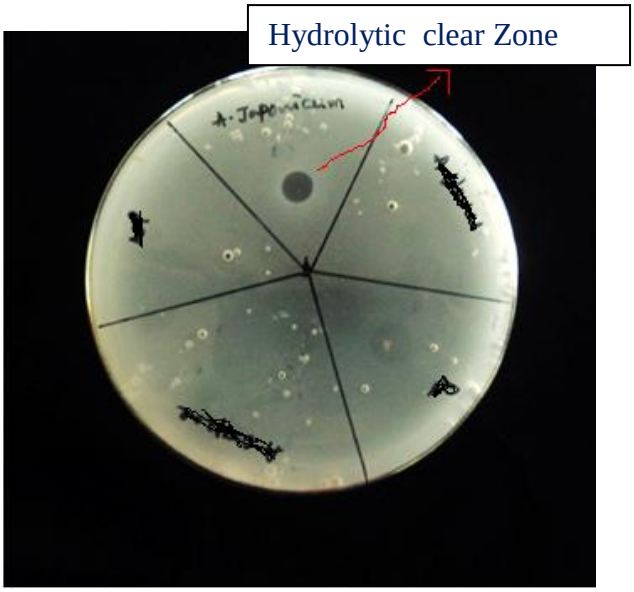

Plate-1: Fibrin plate assay by *Aspergillus japonicum*

Production of fibrinolytic enzyme through submerged fermentation

The isolate was grown in Czapek-Dox media: Composition (g/l) Sucrose-30.0; Sodium nitrate-2.0; K2HPO4-1.0, MgSO4. 7H2O-0.5; KCl-0.5; FeSO4-0.01 for 96 -120h on a shaker with constant 140 rpm. The pH 6, temperature 30^{0}C and 1 ml inoculums size were maintained.

Assay of fibrinolytic enzyme

This was basically measured by the modified method of Anson, 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 [21].

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:

Fungal isolates were isolated from different regions from Bangalore and identified as *Aspergillus japonicum* in Agrakar Research Institute, Pune. All thirty strains of *Aspergillus japonicum* produced clear zone around colony in fibrin plate medium and *Aspergillus japonicum* KSS 05 showed 6 mm diameter of cleared zone those were selected from the soil sample. Of the thirty isolates *Aspergillus japonicum* KSS 05 was considered to be the best and high fibrinolytic enzyme producing strain. It showed 6 mm of cleared zone around the colony (Plate-1). The data obtained in the present study on the effect of pH, temperature and inoculums size on submerged fermentation is shown in Fig.1, which reveals that the production of fibrinolytic enzyme increased with the increase in the pH of the medium up to pH 6.0 temperatures 30^{0}C and inoculum size 1.0 ml, for this it showed 235 U/ml thereafter the decrease of fibrinolytic activity was observed.

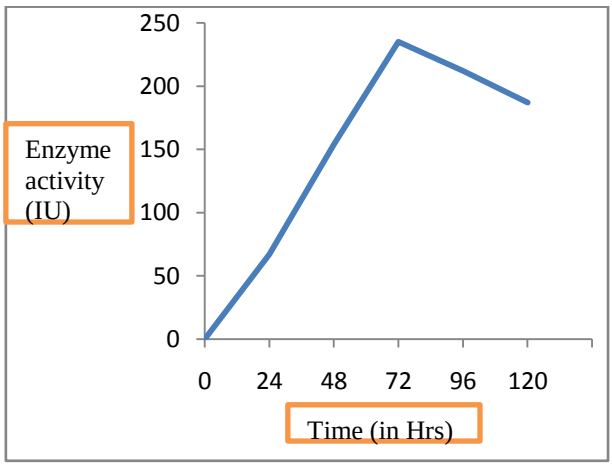

Figure 1: Production of fibrinolytic enzyme by optimized fermentation kinetics

Shilpa et al., [22] were reported that production of fibrinolytic enzyme showed better activity 180U/ml at pH 6 temperature 40^{0}C and inoculum size will be 1.25 ml. Usama et al. [23] showed that incubation temperature 35^{0}C is optimum for maximum production of fibrinolytic enzyme and it showed 2.30 unit/mg protein. Keeping this in view, experiments were conducted to find out the effect of temperature on fibrinolytic enzyme production by *Aspergillus japonicum* KSS 05. It is generally necessary to optimize age and size of the inoculums, because low density gives insufficient biomass and high density produces too much biomass and resulting in depletion of nutrients necessary for fibrinolytic protease production. The earlier studies reported that inoculums size has crucial effect in fermentation process through microorganisms. In our studies 1.0 ml of inoculum size were showed better yield. Venkatanagaraju and Divakar [24] reported that 2 % of mutant *Bacillus cereus* spores as an inoculums from one week old culture were inoculated for the maximum production of fibrinolytic enzyme and our results are consistent with Venkatanagaraju and Divakar [24].

CONCLUSION

Aspergillus japonicum KSS 05 were isolated from different soil samples from different regions from Karnataka and screened for the production of fibrinolytic enzyme by plate assay and also initial fermentation parameters studies were also carried out for the maximum production of fibrinolytic enzyme.

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