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Effect of Mutational Studies on Biosynthesis of Fibrinolytic Enzyme from *Aspergillus japonicum*

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Fibrinolytic enzymes constitute one of the most important groups of industrial enzymes *Aspergillus japonicum* KSS 05 strain were used for fibrinolytic enzyme production. The potential strain were kept for mutational studies. UV radiation is used as mutagenic agent. The mutant obtained i.e *Aspergillus japonicum* KSS 05 *mu* were used for screening of fibrinolytic enzyme production by plate assay and it showed 6 mm diameter clear zone around the colony. Screened mutated strain was employed for the production of fibrinolytic enzyme through submerged fermentation and it showed 252 IU after 72 hr of fermentation. It showed better results compared with *Aspergillus japonicum* KSS 05 parent strain.

Introduction

Cardiovascular diseases (CVDs) have now become the leading cause of mortality in India. A quarter of all mortality is attributable to CVD. Ischemic heart disease and stroke are the predominant causes and are responsible for >80% of CVD deaths. The Global Burden of Disease study estimate of age-standardized CVD death rate of 272 per 100 000 population in India is higher than the global average of 235 per 100 000 population (Prabhakaran *et al*., 2016).

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 have been purified from these foods and their physiochemical properties have been characterized. Fermented shrimp paste, a popular Asian seasoning, was shown to have strong fibrinolytic activity. These novel fibrinolytic enzymes derived from traditional Asian foods are useful for thrombolytic therapy. They will provide an adjunct to the costly fibrinolytic enzymes that are currently used in managing heart disease, since large quantities of enzyme can be conveniently and efficiently produced (Dubey *et al.*, 2011).

The microorganisms producing fibrinolytic enzymes include bacteria, actinomyces, fungi

and algae. Microorganisms are important resources for thrombolytic agents. Streptokinase from *Streptococcus hemolyticus* and Staphylokinase from *Staphylococcus aureus* were earlier proved to be effective in thrombolytic therapy (Collen and Lijnen. 1994). Some kinds of fungi have also been found to produce the protease with high fibrinolytic activity for example *Asperigillus ochraceus* 513, *Fusarium oxysporum*, *Penicillum chrysogenum*, *Rhizopus chinesis* 12. In addition, Matsubara *et al* , found the fibrinolytic enzymes from marine algae *Codiumlatum*, *Codiumdivaricatum*, and *Codiumintricatum* (Shilpa *et al*., 2014).

This work was undertaken to investigate the comparative mutational studies on *Aspergillus japonicum* for fibrinolytic enzyme production in submerged fermentation in a search of eminent production of fibrinolytic enzyme through strain improvement which is desirable.

Materials and Methods

Screening of fungal isolate

Experimental microorganism *Aspergillus japonicum* were isolated from soils collected from different regions in and around Bangalore. The *Aspergillus japonicum* KSS 05 (Plate-1) were isolated by using Czapek Dox's media and shown good zone of clearance on fibrin plate. This strain was selected for mutational studies.

Mutational studies on *Aspergillus japonicum*

The strain *Aspergillus japonicum* KSS 05 (Plate-1) was induced through a physical mutation by subjected to UV radiation for enhancing the production of fibrinolytic enzyme. *Aspergillus japonicum* KSS 05 (Plate-1) spore suspensions were prepared and were irradiated using a 15W Philips UV lamp at various distances (5, 10, 15, and 20 cm) for 15 min (Siddalingeshwara *et al*., 2010). The irradiated spore suspensions were inoculated on Czapek Dox agar plates. The present study highlights with the parent and mutant strain which was isolated. The strain was labelled as *Aspergillus japonicum* KSS 05 mu and used for production of fibrinolytic enzyme.

Comparative studies on Parent and Mutant strains of *Aspergillus japonicum* KSS 05 Inoculum

The spore suspension was prepared by adding 10mL of 0.01% Tween 80 solution to 168h fresh culture slant and was suspended the spores well with the sterile loop which was used as a inoculums (Lingappa and Vivek Babu, 2005). 1mL of spore suspension of inoculum contains a final concentration of 1 x 10^7spores/ml.

Fermentation Medium

The prepared inoculum of *Aspergillus japonicum* KSS 05 was added to the fermentation medium. The fermentation medium composition is (g/l) Sucrose-30.0; Sodium nitrate-2.0; K_2HPO_4-1.0, $MgSO_4$. $7H_2O$-0.5; KCl-0.5; $FeSO_4$-0.01 for 96 -120h on a shaker with constant 140 rpm. The pH 6, temperature 30°C and 1 ml inoculums size were maintained..

Submerged fermentation

250mL Erlenmeyer flasks were used for submerged fermentation. 100mL of fermentation medium was prepared in each flask and autoclaved at 121°C, 15lbs for 20 min and cooled to room temperature. Then 1 mL of freshly prepared inoculum suspension was inoculated aseptically in each one and the broth was thoroughly mixed and incubated for 72 to 120h at 30°C.

Extraction of fibrinolytic enzyme

The culture media samples were extracted of volume approximately 5 mL at 24 h of successive intervals aseptically. The extract was filtered through Whatman filter No. 1. The filtrate was centrifuged at 2000-3000 rpm for 15 min, supernatant were used for enzyme preparation. Thus prepared crude enzyme was used for assay of fibrinolytic enzyme.

Assay of fibrinolytic enzyme

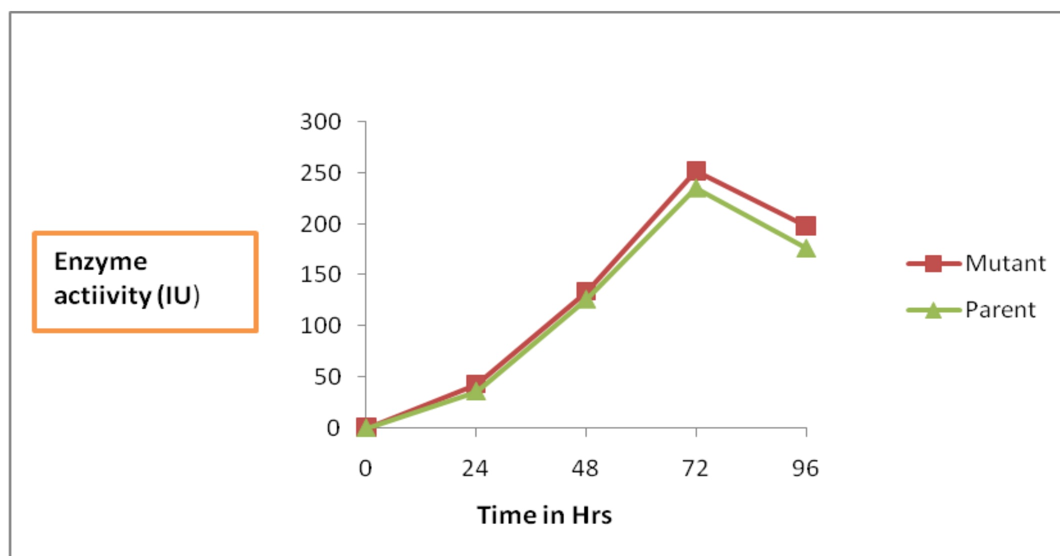
This was basically measured by the modified method of Anson, but with a few modifications. The reaction mixture contained 1 ml of 1.2% of bovine fibrin solution in Tris-HCl buffer (pH 8.0) and 1 ml of cell free supernatant (CFS). The reaction mixture was incubated for 2 h at 37°C. Then the reaction was 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 (Mukesh Kumar *et al*., 2013).

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 Discussions

In present study, thirty strains were isolated and screen for the production of fibrinolytic enzyme by plate assay. *Aspergillus japonicum* KSS 05 was selected as it was the potent fibrinolytic enzyme producer. It exhibited 6 mm diameter of zone of clearance and 39.15% of hydrolysis around the colony. This potent strain was used for mutational studies. Because the need of selecting a suitable strain is immensely demanded that is capable of producing commercially acceptable yield for bioprocessing.

Fig.1 Effect of Mutational studies on Biosynthesis of Fibtinolytic enzymes



The mutant strain *Aspergillus japonicum* KSS 05 *mu* was also kept for biosynthesis of fibrinolytic enzyme and the strain which was kept at a distance of 15cm from UV lamp showed better fibrinolytic activity. Furthermore, both the parent and the mutant strains were used to produce fibrinolytic enzyme in fermentation medium. The mutant *Aspergillus japonicum* KSS 05 *mu* was showed the highest production of fibrinolytic

enzyme of 252 IU at 72h fermentation period in submerged fermentation (Fig-1). While comparing with mutant, the parent strain *Aspergillus japonicum* KSS 05 could yield only 235 IU at 72h fermentation period. The mutagenic irradiations, usually X-ray, gamma rays and UV rays are used to target fungi. Because the wavelength and penetrating power was high for X-ray and gamma ray, lead to greater unclear damages which lead to lethal effects in target organisms (Rani and Prasad, 2012).

They are powerful mutagens cause damage of nucleus, so they are avoided. Therefore, mild mutagens like UV rays with shorter wave length than visible light are considered to be ideal for mutational irradiations (Rani and Prasad, 2012). Venkata Naga Raju and Goli. Divakar (2013) were reported on physical mutation for biosynthesis of fibrinolytic protease. The isolates were sub grouped into G-I (8-11 U/ml/min), G-II (11.1-13 U/ml/min) and G-III (more than 13.0 U/ml/min) according to their enzyme productivity. Of all the isolates investigated, maximum enzyme production (14.60 ± 1.15 U/ml/min) was obtained by GDA17 which was selected after 70min of UV treatment. The production of enzyme following the growth of the organism was found to be highly significant than other mutant derivatives. Our results are best agreements with the results of Venkata Naga Raju and, Goli. Divakar (2013).

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