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EFFECT OF METAL IONS FOR THE BIOSYNTHESIS OF FIBRINOLYTIC ENZYME FROM *ASPERGILLUS TAMARI*

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ABSTRACT

Thrombolytic agents are used to treat heart attack, stroke, deep vein thrombosis, pulmonary embolism and occlusion of a peripheral artery or in dwelling catheter1. All thrombolytic agents are serine proteases and convert plasminogen to plasmin (EC 3.4.21.7), which breaks down the fibrinogen and fibrin and dissolves the clot. Fibrinolytic enzyme production was investigated in the filamentous fungi *Aspergillus tamarii* SAS 02 a soil isolate from different regions of Karnataka and produces 152 IU. The process economization were employed to achieve higher yield of fibrinolytic enzyme through submerged fermentation (SmF) and here we made an attempt to incorporate metal ions as a trace elements sources such as copper sulphate ($CuSO_4$), iron sulphate ($FeSO_4$), Zinc sulphate ($ZnSO_4$) and Magnesium sulphate ($MgSO_4$) source to the production medium. In case of zinc sulphate the maximum fibrinolytic enzyme production of 152 IU was observed at 0.02% and where as copper sulphate ($CuSO_4$), iron sulphate ($FeSO_4$) and magnesium sulphate ($MgSO_4$) yielded maximum fibrinolytic enzyme of 71 IU, 98 IU and 95 IU.

KEYWORDS: Fibrinolytic enzyme, metal ions and *Aspergillus tamarii*.

INTRODUCTION

The cardiovascular diseases have become the leading cause of death in the Western world (Viles et al., 2004). Many blood clot-dissolving agents, such as urokinase, streptokinase and tissue plasminogen activator (t-PA), have been utilized in clinical treatments for cardiovascular diseases. Hemostasis is a complex process obtained through an optimal balance between bleeding and blood clot formation. In an unbalanced state, fibrin clots may not be lysed resulting in thrombosis.

Disorders of blood clotting and fibrinolysis are serious medical problems. Thrombosis, which is particularly serious, can lead to cerebral and myocardial infarction due to un-lysed blood clots (Holden 1990). 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 (Takeno *et al*. 1999).

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).

Present study will highlights on the biosynthesis of fibrinolytic enzymes from *Aspergillus tamari.*

MATERIALS AND METHODS

Fungal isolation and identification

Aspergillus tamarii SAS 02 strain were isolated from different soil samples. Soils are taken from different from regions from Karnataka, such as Tumkur, Bangalore and Bijapur. and and confirmed by molecular level identification.

Screening and molecular identification of fibrinolytic enzyme producing *Aspergillus tamari*

Aspergillus tamari were used to screen by fibrin plate assay (Astrup and Mullertz, 1952). The identification of *Aspergillus tamarii* were carried out by molecular level. Initially DNA were extracted from fungi and Amplification of the ITS region were carried out by

using ITS Primers (5' to 3'). Both strands of the rDNA region amplified by PCR were sequenced by automated DNA sequence -3037*xl* DNA Analyzer. Sequences were compared to the non-redundant NCBI database using BLASTN.

Effect of Metal ions as source for the biosynthesis of fibrinolytic enzyme

A set of conical flasks with 100ml of production medium supplemented with a particular metal ions as a source such as copper sulphate ($CuSO_4$), iron sulphate ($FeSO_4$), Zinc sulphate ($ZnSO_4$) and magnesium sulphate ($MgSO_4$) were used for screening their effect on production of enzyme at 0.001 and 0.003% in production media devoid of $MgCl_2$. The culture was grown for 96 h with enzyme assay at every 24h. The production medium consists (mg/100ml) of Sucrose 3, di potassium hydrogen phosphate 0.1, KCl 0.05g, NaCl and MgSO4 and FeSO4 are in the range of 0.001 and 0.003%. The condition of the fermentation medium is as fallows. pH,6 temperature 40^0C and inoculums size is of 1.25ml.

Extraction of fibrinolytic enzymes

The samples were withdrawn periodically at 24hrs in aseptic condition. The extract was filtered through What'sman filter No.1. The clear extract was centrifuged at 2000-3000rpm for 15min, 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 (1939), 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 2ml 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 750nm (Mukesh Kumar, et al., 2013).

Unit

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

RESULTS AND DISCUSSION

The *Aspergillus tamarii* SAS 02 were isolated from soil sample and screened for the fibrinolytic enzyme production and the fungal isolate were identified by molecular level by amplification and sequencing were carried out and finally phylogenitic studies were consider for the confirmation of *Aspergillus tamarii*.

The influence of metal ions for fibrinolytic enzyme production were carried out with concentration of 0.001 and 0.003%. The results revealed that all the metal ion sources employed under the present study have enhanced the production of fibrinolytic enzyme up to 0.02% of metal ions at 72hrs of fermentation represented in Fig1-4, thereafter no significant production of fibrinolytic enzyme was observed on all the days of fermentation period. In case of zinc sulphate the maximum fibrinolytic enzyme production of 152 IU was observed at 0.02% and where as copper sulphate ($CuSO_4$), iron sulphate ($FeSO_4$) and magnesium sulphate ($MgSO_4$) yielded maximum fibrinolytic enzyme of 71 IU, 98 IU and 95 IU.

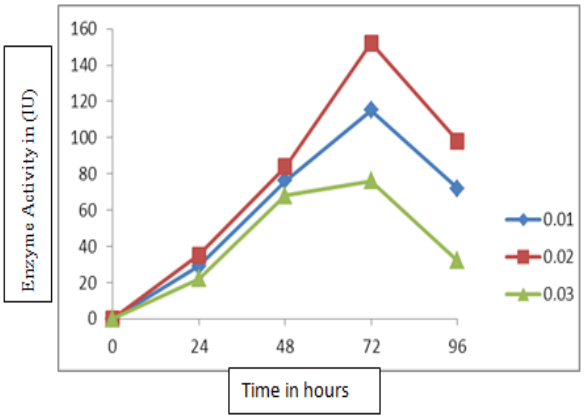

Fig.1: Effect of Zinc sulphate on fibrinolytic enzyme production.

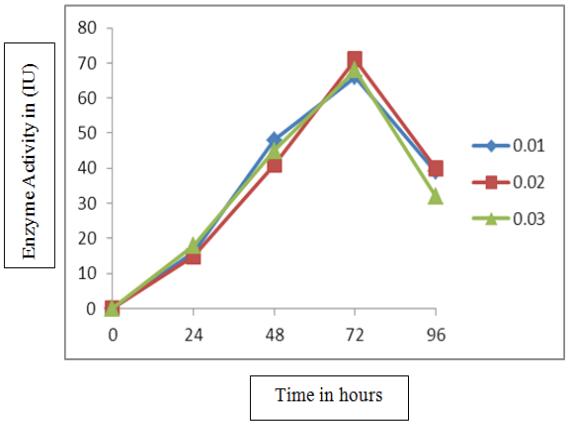

Fig.2: Effect of Copper sulphate on fibrinolytic enzyme production.

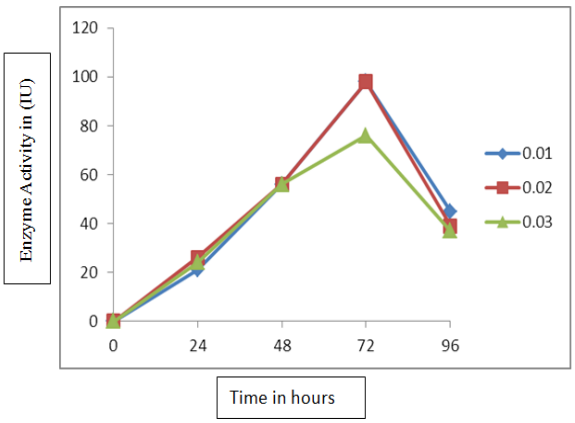

Fig.3: Effect of Iron sulphate on fibrinolytic enzyme production.

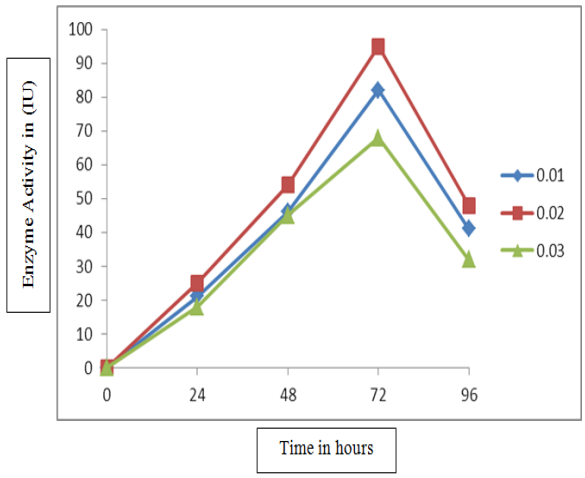

Fig.4: Effect of Magnesium sulphate on fibrinolytic enzyme production.

Trace elements have profound effect on the growth and physiological activities of the organisms. In general trace elements play a key role in the metabolism of organisms. Few metal ions need to be supplemented to a fermenting medium, as they are essential for cell mass formation and also acts as a co factor for several biosynthetic enzymes.

Abdel-Naby et al., (1991) were reported on Adding trace metals (Zn2+, Fe^{H}, Mn^{H}), separately or combined, to the medium had no significant effect on the production of fibrinolytic enzyme by using *Streptomyces* sp, NRC 411. Deepak et al., (2008) were highlighted on supplementation of MgSO4–0.2% and CaCl2–0.5% increased and stabilized the Nattokinase activity of the enzyme; this is possible because of the activation by the metal ions. Usama F. Ali and Ibrahim (2006) reported that, they supplemented magnesium sulphate (0.5g/l), potassium chloride (0.5g/l) and iron sulphate (0.01g/l) by using *Aspergillus tamarii* for the synthesis of fibrinolytic enzyme synthesis and it synthesized 1.71 IU/ml. Our results were coincides with the Usama F. Ali and Ibrahim (2006).

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