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RESEARCH ARTICLE

Comparison of Streptokinase Activity from *Streptococcus mutans* using Different Substrates

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ABSTRACT

Streptokinase is a novel bacterial fibrinolytic enzyme that binds and activates plasminogen and is produced by several species of *Streptococci*. *Streptococcus mutans* was selected for optimum production of streptokinase using corn steep liquor, molasses, rice polishing and sugarcane bagass in liquid state fermentation. Substrates were applied in different concentrations ranging from 0.1-0.8%. Maximum fibrinolytic activity was observed by 0.3% corn steep liquor, 0.5% molasses and rice polishing and 0.4% by sugarcane bagass. The fibrinolytic activity achieved by fibrin clot lysis method was 5.5, 5.08, 5.16 and 4.75 units using corn steep liquor, molasses, rice polishing and sugarcane bagass, respectively.

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INTRODUCTION

Streptokinase has wide applications in human and animal species, where it is the drug of choice to lyse the blood clot in vessels. There are many reports, where streptokinase has been used as an effective drug in case of thrombosis, arterial thromboembolism and critical stenosis in various animals like cats, rats, rabbits and dogs (Moore *et al*., 2000; Vlasin *et al.,* 2005). The use of streptokinase as a thrombolytic agent, especially in the treatment of acute myocardial infarction in human is also well documented since after its discovery in 1933. The occurrence of 17 million cardiovascular deaths per annum and 32 million annually reported heart attacks are enough to draw attention to the scale of this epidemic in the present world. In developing countries, like Pakistan, the frequency of cardiac deaths is at alarming stage. Streptokinase is currently used in clinical medicine as a therapeutic agent in the treatment of thromboembolic blockages, including coronary thrombosis in animals and human as well.

Streptokinase (SK) is naturally formed and secreted by various strains of hemolytic *Streptococci* (Babashamsi *et al*., 2009). It is an extracellular protein composed of 414 amino acids with a molecular mass of 47 kDa. It is a non-protease plasminogen activator that activates plasminogen to plasmin, the enzyme that degrades fibrin clot through its specific lysine binding site. Therefore, it is used as a drug in thrombolytic activity (Babashamsi *et al*., 2009). Unlike urokinase or tissue-type plasminogen activators, that perform direct proteolysis, SK forms a high affinity equimolar complex with plasminogen (Dubey *et al*., 2011). It is now the leading fibrinolytic agent in the treatment of thromboembolic conditions (Boersma *et al*., 2003).

In normal circulation, clot formation is inhibited by a healthy and normal hemostatic system. Pulmonary embolism, deep vein thrombosis, stroke, acute myocardial infarction and ultimately death could be the results of an unsuccessful haemostatic system. Hemostasis is a versatile process achieved through an optimal balance among hemorrhage and blood clot formation. Fibrin clots may not be lysed resulting in thrombosis, in an unbalanced situation. Previously, management of thromboembolic vascular diseases was relied on the use of anti-coagulants so to inhibit the formation of fibrin clot. Fibrin lysis can take place *in vivo* by alteration of plasminogen to plasmin (Balaraman and Prabakaran, 2007).

Comparative clinical trials in the treatment of acute myocardial infarction suggested that streptokinase is a cost-effective and useful thrombolytic drug for both animal and human disorders (Banerjee *et al*., 2004). Hence, several studies have been conducted focusing on

production and improvement of streptokinase (Zhai *et al*., 2003). Therefore, the main objective of this research study was to obtain optimum yield of SK enzyme with better fibrinolytic activity using *Streptococcus mutans* in liquid state fermentation using optimum CSL, molasses, rice polishing and sugarcane bagass concentrations.

MATERIALS AND METHODS

Streptococcus mutans, obtained from Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan was selected as test organism for streptokinase production in liquid state fermentation. Bacterial growth was recognized by blood agar media and the isolates exhibiting streptokinase activity were used in further studies.

Enzyme production: To seek the suitable substrate at its optimum level, CSL, molasses, rice polishing and sugarcane bagass were selected as the substrates for streptokinase production (Rasul *et al.,* 2011). *Streptococcus mutans* was grown on medium containing peptone 2%, beef extract 0.5% and NaCl 0.5% at pH 7.0. A loopful culture of *Streptococcus mutans* was transferred aseptically into the medium; incubated at 37^{o}C and 120 rpm in an orbital shaker for 24 hours (Patel *et al.,* 2011). Then, 2.5% of culture was used as inoculum in a sterilized liquid state fermentation media containing yeast extract 2%, glucose 2%, KH_2PO_4 0.05%, $CaCO_3$ 0.005% and substrate (CSL, molasses, rice polishing and sugarcane bagass) in different concentrations ranging from 0.1-0.8%. Streptokinase enzyme was harvested from biomass after 24 hours by filtration and then centrifugation at 10,000 rpm for 20 minutes at 0^{o}C. Enzyme was stored at -20^{o}C and supernatant was assayed for enzyme activity (Dubey *et al*., 2011).

Enzyme assay: One unit of streptokinase is an amount of enzyme converting 1 micro-mole of substrate in one minute. Streptokinase assay is dependent upon the activation of plasminogen to plasmin that hydrolyzes the substrate in a defined period which is linked back to concentration of streptokinase. A modification of Holmstrom method (Holmstrom, 1968) was used where 1 mL of fibrinated blood was taken equally in 2 test tubes and added 0.25 mL of enzyme extract at 37^{o}C, for its lysis in a unit time. According to Madhuri *et al*. (2011), 1 mL of human clotted blood is completely lysed by 0.12 mL of enzyme solution, known as 1 unit.

Statistical analysis: Mean was used for the comparison of enzymatic activity of optimum substrate concentration whereas ANOVA was used for the determination of significance of the results.

RESULTS

Substrate Optimization: *Streptococcus mutans* was grown in submerged fermentation using different concentration of CSL ranging from 0.1-0.8% as substrate. Maximum streptokinase enzymatic activity (38.6%) was observed at 0.3% that was selected as the optimum concentration of CSL for streptokinase production (Fig. 1).

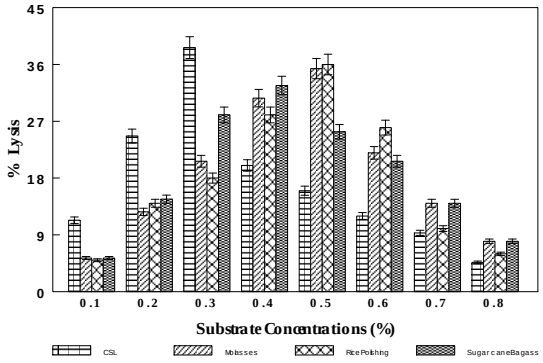

Fig. 1: Activity of streptokinase using different substrates at various concentrations

Among different concentrations of molasses maximum fibrinolytic activity was obtained by streptokinase enzyme produced using 0.5% concentration of molasses showing 35.3% lysis (Fig. 1). Thus, it was selected as the optimum concentration for production of streptokinase.

Among different concentrations of rice polishing the highest fibrinolytic activity was observed, when *Streptococcus mutans* was grown for streptokinase enzyme production in liquid state fermentation using 0.5% concentration of rice polishing as substrate that showed 36% lysis (Fig. 1). Therefore, 0.5% concentration of rice polishing was selected as optimum substrate concentration for streptokinase production by *Streptococcus mutans* as enzyme producing microorganism in liquid state fermentation.

Streptokinase enzyme produced by *Streptococcus mutans* species grown using 0.4% concentration of sugarcane bagass in liquid state fermentation, indicated 32.6% highest fibrinolytic activity (Fig. 1). Consequently, 0.4% concentration of sugarcane bagass was selected as optimum concentration for streptokinase production.

Comparison of optimized substrate concentrations with standard enzyme: After the optimization of different substrates, 0.3% concentration of CSL, 0.5% molasses and rice polishing and 0.4% sugarcane bagass, were selected as the optimum concentrations in liquid state fermentation for streptokinase production. Afterwards, experimentally selected optimum concentrations of CSL, molasses, rice polishing and sugarcane bagass were used to grow *Streptococcus mutans* in liquid state fermentation in order to compare the best substrate with standard streptokinase enzyme. As compared to 7 U of activity obtained by standard streptokinase; it was found that 5.5 U, 5.08 U, 5.16 U and 4.75 U were observed by optimum concentrations of CSL, molasses, rice polishing and sugarcane bagass, respectively.

DISCUSSION

There are many thrombolytic agents including streptokinase, urokinase, tissue plasminogen activator and various antithrombic agents that may be used to treat cardiovascular disorders in human and animals as well. Killingsworth *et al.* (1986) conducted a study to treat aortic thromboemboli with streptokinase in cats and

reported that the cats revealed absolute thrombus dissolution. Moore *et al.* (2000) applied SK on 46 cats suffering from arterial thromboembolism and found that 25 cats recovered the pulses within 2-24 hours of SK administration. It also showed a 33% short term survival rate, while other subjects were improved after a bit longer time periods. In another study (Vlasin *et al.*, 2005), rabbits, having primary thrombolysis, treated with SK showed a 7% decline in size of thrombus with a reduction of 64% radioactivity of the original thrombus.

Tissue plasminogen activator (tPA) is an alternative choice for thrombolytic treatment in human and animals like rabbits and cats. But it is proved and well understood that tPA is more costly than streptokinase (Moore *et al.*, 2000). This article describes the production of fibrinolytically active enzyme streptokinase in liquid state fermentation and effect of various substrates on fibrinolytic activity of streptokinase produced using *Streptococcus* species. As mentioned above, intravenous administration of streptokinase has been widely used for thrombosis therapy. So, optimal experimental methodology should be well defined for its high yield such as optimum substrate concentrations, pH controlling agents and optimum temperature ranges etc. Lee *et al.* (1997) reported that microbes are very effective and useful sources for enzyme production. Thus, for assessment of streptokinase application as a thrombosis agent, microbes such as *Streptococcus* are preferred for its production. Therefore, *Streptococcus* species for streptokinase production in liquid state fermentation was used. According to Dubey *et al*. (2011), the fibrinolytic activity of streptokinase obtained from *Streptococcus* species using 8% CSL was 2.5 U, but in contrast to present study, the enzyme activity was much better. By using only 0.3% CSL concentration fibrinolytic activity was 5.5 U. In addition, 5.08, 5.16 and 4.75 U fibrinolytic activity was obtained using only molasses (0.5%), rice polishing (0.5%) and sugarcane bagass (0.4%), respectively. It indicated economical and more favorable findings for streptokinase production at industrial scale. In view of the present study, it may be suggested that fibrinolytic enzyme streptokinase may be produced in liquid state fermentation and different easily available and commonly used substances such as CSL, molasses, rice polishing and sugarcane bagass can be used as substrates in their optimized concentrations for better and high yield of enzyme.

Conclusion: Streptokinase is being used as thrombolytic agent for livestock and human health care but due to high cost and its intravenous installation, large scale production by some alternative sources and methods is required. Therefore, streptokinase production using bacterial sources i.e., *Streptococcus mutans* is very effective and useful. We reported that streptokinase having better fibrinolytic activity can be produced by using optimal concentration of CSL and other substrates to fulfill the needs of this enzyme in our country.

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