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Isolation, Production And Purification Of Streptokinase From Streptococcus Pyogenes

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Abstract: Streptokinase a fibrinolytic enzyme is very effective in treating acute myocardial infarction and it is certainly more cost-effective. In view of the relatively recent availability of the competing recombinant tPA, skepticism is being expressed about the continued viability of streptokinase therapy. Despite this research on streptokinase continues, and it remains a vital and affordable therapy especially in the world's poorer healthcare systems. Our present study focuses on the production of streptokinase from Streptococcus pyogenes species and partial purification of streptokinase by ammonium sulfate precipitation, dialysis and column chromatography. The enzyme is quantified by Lowry's method and its electrophoretic mobility and molecular weight were determined by SDS-PAGE.

Keyword: Streptokinase, Streptococcus pyogenes, Tissue Plasminogen Activator, SDS-PAGE.

INTRODUCTION

The clinical importance of streptokinase was first noted by Tillet and Garner, who discovered that this bacterial protein caused the lysis of human blood clots. It was later found that streptokinase is not an enzyme but rather a potent activator of plasminogen, the inactive precursor of plasmin. Plasmin is the active fibrinolytic component of the circulatory system, solubilizing the fibrin network in blood clots through limited proteolysis. Streptokinase is currently used in clinical medicine as a therapeutic agent in the treatment of thromboembolic blockages, including coronary thrombosis Streptokinase is naturally produced and secreted by various strains of hemolytic streptococci. The best studied of these is the streptokinase from Streptococcus equisimilis, from which the secretion of streptokinase into the external medium is directed by a 26 amino acid signal peptide which is cleaved during the secretion process. The mature protein has a molecular weight of about 47 kilo Dalton (KD) and was found to be composed of 415 amino acid residues. Karush, Iacocca, and Harris and Ogburn, Harris, and Harris studied the growth of a β-hemolytic streptococcus in continuous culture with pH as a limiting factor. In these experiments, pH was controlled only by addition of buffer to the medium. The yield of cells and of some extra cellular antigens was investigated. Rosenberger and Essen studied the effect of both glucose and tryptophan limitation on growth in continuous cultures of a Streptococcus faecalis strain. Their findings indicate that, to obtain maximal cell yield per unit energy source, the energy source should be the limiting factor. Several methods have been reported for the purification of streptokinase obtained from the culture media of various strains of streptococci. In some cases DEAE-cellulose has been used in combination with other purification procedures and a highly purified product has been obtained. Other chromatographic procedures have also been used for the purification of streptokinase by combining more than one purification step. Castellino et al reported the use of affinity chromatography on immobilized Di-Isopropyl phosphate (DIP)- plasmin for single step purification of streptokinase. This method involved the conversion of plasminogen to plasmin by urokinase and the inhibition of plasmin protease activity by diisopropyl fluorophosphates. Jeong et al reported an affinity chromatography using plasminogen as a ligand. Recently we have produced a fusion recombinant streptokinase and purified it in a single step affinity chromatography using glutathione as the ligand. In this paper we are reporting the results obtained from isolation of streptokinase producing strain, production of streptokinase and its purification by four different techniques i.e centrifugation, ammonium sulfate precipitation, dialysis, ion exchange chromatography and purity testing by native PAGE

2.MATERIALSANDMETHODS

2.1 Isolation and screening of microbial strain

Blood Agar medium was prepared. After solidification the plates were kept at room temperature to check contamination.

Screening of microbial strain

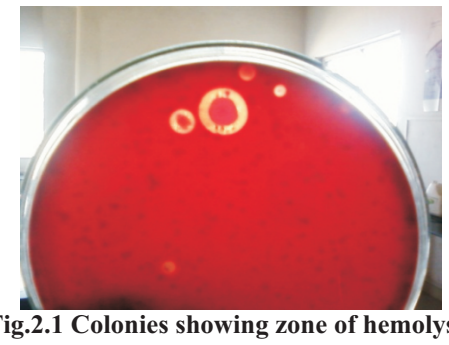

Fig.2.1 Colonies showing zone of hemolysis

The Blood Agar plates were showing number of colonies some of which shows zone of hemolysis i.e. those colonies were able to digest fibrin present in the media. From these blood agar plates 5 Colonies selected and named as A, B, C, D and E respectively. Confirmation of microorganism was done with blood agar medium, Todd Hewitt broth medium, gram staining and biochemical tests.

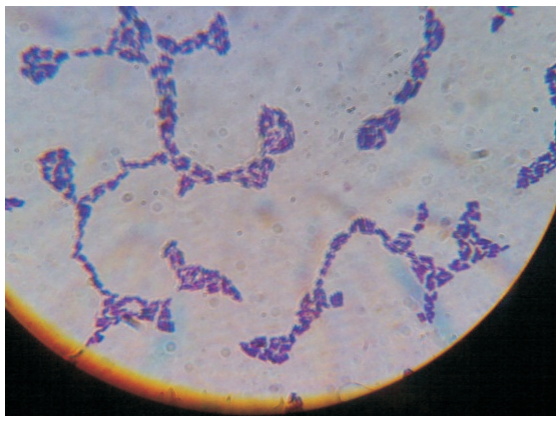

Fig.2.2 Gram staining of isolates

Radial Caseinolysis Assay (Yanjun duan et al. 1998)

The skim milk agar medium was prepared and wells were punctured in agar plate. 25 µl of streptokinase enzyme was loaded into the wells and kept for incubation at 37°C for 12 hours

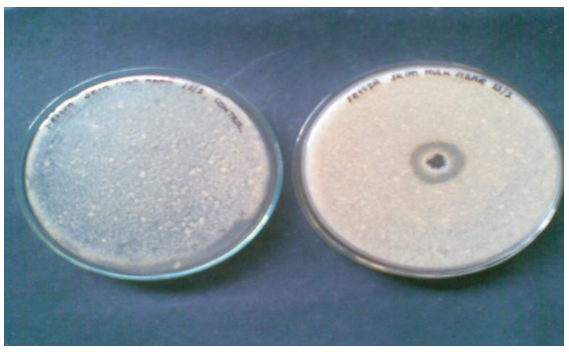

Fig.2.3 Radial Caseinolysis Assay

2.2 Streptokinase production

The bacteria were grown in 25 ml of nutrient broth at 37 °C. By increasing the turbidity to the level of OD-0.6 at 600 nm, it was sub-cultured in 250 ml of broth. It was observed that the optimum pH for cell growth and streptokinase activity was at the neutral condition (pH-7). The fermentation media was inoculated with the 10% inoculum and incubated for 72 hours at 37 oC in shaking incubator at 120 rpm. (Baewald et. al. 1975)

2.3 Purification of streptokinase

2.3.1. Ammonium sulfate precipitation

The cell debris was removed by centrifugation and resultant supernatant was used as the enzyme source. Ionic strength of this supernatant was increased with increase in the pinch wise addition of ammonium sulfate (20%-80%) saturation and resultant precipitation was obtained by centrifugation at 10000 rpm for 10 min at 5oC .This precipitate was dissolved in distilled water and used as a crude source of enzyme.

2.3.2. Dialysis:

A Protein solution was placed in dialysis tubing to remove the low molecular weight proteins at room temperature for 2 days. After dialysis, the streptokinase activity as well as protein content were measured.

2.3.3. Anion exchange chromatography.

Dialyzed protein solution was further purified on to DEAE-cellulose (De Renzo et al. (1967). The dialyzed enzyme was applied on the activated DEAE-Cellulose column (1.5x15 cm) that was pre-equilibrated with sodium phosphate buffer [0.01M pH-7.0]. Gradient elution was carried out using NaCl solutions of different molarities. five fractions were collected by the each molar concentration of NaCl solution at the flow rate 1ml/min. Each fraction contained 3.0 ml solution and checked for the streptokinase activity as well as protein content.

2.3.4. Confirmation of purity of streptokinase by native-page
The purity of streptokinase was determined by separation on NATIVE-PAGE.

3.RESULT AND DISCUSSION

3.1. Purification by centrifugation

The fermented broth was centrifuged at 10000 rpm for 15 min at 5oC temperature. As the enzyme is extra cellular the supernatant was taken. Hence Crude enzyme was extracted successfully and further purified by dialysis.

3.2. Ammonium sulfate precipitation

The crude enzyme solution was extracted with distilled water at optimum condition and precipitation of streptokinase from the aqueous extract by ammonium sulfate (NH4)2SO4 (20-80) % Showed good recovery in their activities (60%),while saturation with 20-50%and 70-80% (NH4)2SO4 resulted in precipitate with low enzyme activity & high protein content ,therefore these precipitate require further purification.

3.3. Dialysis This crude sample dialyzed overnight for 2 days .The protein was concentrated and unwanted protein were eliminated.

3.4. Anion exchange chromatography

Purification of streptokinase by using DEAE-Cellulose as an anionic exchanger shows the elution of protein at 0.2M NaCl gives the better result than that of the 0.1M, 0.3M, (elution buffer). Finally the estimation of protein was carried out by Folin-Lowry method showing blue color formation in the protein sample eluted from the chromatographic column by using eluting buffer of 0.2M sodium NaCl buffer.

3.5. Purity determination by gel electrophoresis

The purified streptokinase migrated as two isolated bands on Native-PAGE indicating streptokinase may be possess the isoforms.

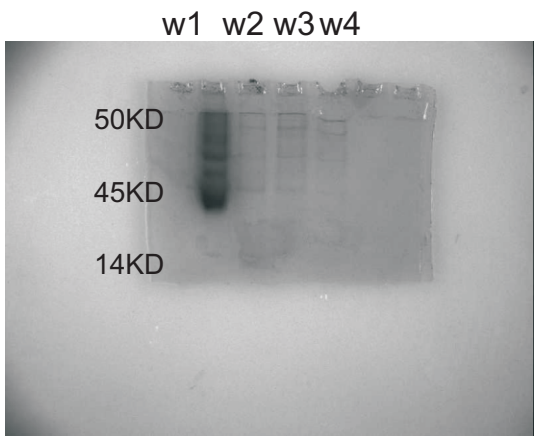

Fig.3.1 Native PAGE

Where, Wells 1, 2, 3, 4 contains protein marker, crude sample, dialyzed sample and Sample after ion exchange chromatography respectively.

4. CONCLUSIONS

Streptokinase is used as thrombolytic agent but too costly and also used through intravenous instillation, needs large scale production by some alternative methods and high purity. So, isolation, production and purification of fibrinolytic enzymes from bacterial sources are very effective and useful. In the future, the research will progress into the production of highly purified fibrinolytic enzymes from bacterial sources

5. FUTURE PERSPECTIVES

Streptokinase may find a use in helping to prevent postoperative adhesions, a common complication of surgery, especially abdominal surgery (appendectomy, gall stoneshysterectomy, etc.) One study using animal models (rats) found that when used with a PHBV membrane drug-delivery system, it was 90 percent effective in preventing adhesions.

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