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Review Article

Fibrinolytic Enzymes : A Substantial Approach

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Abstract

In this article an attempt is made to review different types of fibrinolytic enzymes from different sources, its production and application. The study reveals fibrinolytic enzymes such as Staphylokinase, Nattokinase, and Streptokinase can act as a potent thrombolytic agent. These agents can be useful therapy for dissolving blood clots by activating plasminogen to plasmin, which further act on fibrin clots. These enzymes have been isolated from *Staphylococcus aureus, Bacillus subtilis, Streptococci* etc. There are different works carried on to make the production of fibrinolytic enzymes easy, cost effective using cheap substrate such as cowdung, agricultural waste etc. Work on fibrinolytic enzymes can reduce the harmfulness of other thrombolytic agents such as EDTA and heparin by increasing its activity and shell life.

Keywords : Staphylokianse, Nattokinase, streptokinase, tissue plasminogen activator and thrombolytic agents.

Introduction

Cardiovascular diseases are one the most important cause of mortality worldwide. Intra-vascular thrombosis due to the accumulation of blood clot in the blood vessels is the main cause of cardiovascular diseases (Dubis and Witkiewicz, 2010). There is many research available involving screening and isolation of micro-organism producing enzyme with high fibrinolytic activity, because most of the commercially available enzymes have complication such as, bleeding, allergic, re-occlusion etc (Chitte, 2013). Fibrino-lytic protease is well-known as a subclass of protease, which has an ability to degrade fibrin (Fujita *et al.,* 1993). In balanced condition, fibrins are hydrolyzed by plasmin to avoid thrombosis in blood vessels. During unbalanced condition, deposition of fibrin in blood vessels increases thrombosis, cardiovascular diseases such as stroke, heart attack, acute myocardial infraction, and atherosclerosis, endometriosis, complication in pregnancy and development of neoplastic disease etc (Voet and Voet, 1990). Fibrinolytic enzymes convert plasminogen to plasmin and lyse clots by breaking down the fibrin contained in the clot. Figure-1 Depicts the pathway of fibrinolysis. Novel fibrinolytic enzymes derived from micro-bial source are useful for thrombolytic therapy (Koide *et al.,* 1982). On the basis of catalytic mechanism microbial fibrinolytic enzymes have been classified into three types: serine protease, metallo protease, both serine and metallo-protease (Rajagopalan *et al.,* 1985).

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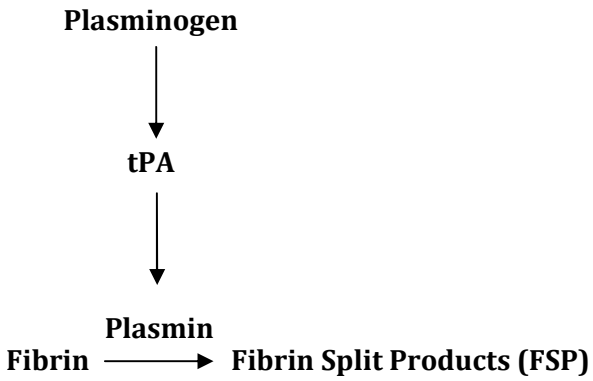
FIBRINOLYSIS



Sources

Microorganisms act as an important source of thrombolytic agents. In the last decades, fibrinolytic enzymes from various microbes have been discovered in succession, such as Streptokinase from *Streptococcus megaspores*, Staphylokianse from *Staphylococcus aureus*, Nattokinase from *Bacillus natto* (Sumi *et al.,* 1987; Collen and Lijnen, 1994). Fibrinolytic producing organisms include bacteria, actinomycetes, fungi and algae (Chitte and Dey, 2000). Fibrinolytic enzymes have been isolated from different sources and have been proved as effective thrombolytic agents. Fibrinolytic enzymes has been screened and identified from Malaysian fermented seafoods such as shrimp paste, fish and shrimp sauce (Zakaria *et al.,* 2015). Nattokinase producing *Bacillus* sp. CK from the Korean fermented soybean sauce named Chungkook-Jang (Kim *et al.,* 1996). Staphylokinase producing *S. aureus* isolated from clinical sources and environmental samples (Rana *et al.,* 2016; Subathra Devi *et al.,* 2012). Streptokinase from patient suffering from sore throat, scarlet fever and acute tonsillitis (Gull-E-Faran *et al.,* 2015). Fibrinolytic protease producing mesophilic bacteria from the slaughter house (Raju and Divakar, 2013). Therefore, continuous efforts have been focused in the search of safer and less expensive thrombolytic agents from diverse sources. Investigation of extracellular proteases is of great concern in enzymology due to their wide application in clinical, pharmaceutical, food and bioremediation process (Venkata and Divakar, 2015).

Production

The cost of enzyme production and downstream processing is the major obstacle against the successful application of enzymes in industry (Essam *et al.,* 2012). Many attempts are made to improve the expression of fibrinolytic enzymes, including selection of ideal cultural medium, optimization of environmental conditions and over expression by genetically engineered strains. It is necessary to optimize nutrient components and environmental conditions for effective cell growth and production of fibrinolytic enzymes (Lee *et al.,* 1999). For example, sucrose, soya flour and di potassium hydrogen phosphate was found to be the best carbon, nitrogen and mineral source (Anjana *et al.,* 2015). Fibrinolytic protease was produced by solid state fermen- tation using agro industrial substrates. They found that the optimum temperature for enzyme production was 45°C and its original activity retained after it was subjected to 80°c for 120 min (Nascimento *et al.,* 2004). Various nitrogen sources such as beef extract, peptone, soy peptone and milk casein was used for the production of fibrinolytic enzyme and found that the enzyme activity was increased when plasminogen was added as substrate as compared to control. It was also found that fibrin was found to enhance the enzyme production, suggesting that fibrin, as a substrate of fibrinolytic enzyme, could induce or activate enzyme production during cultivation (Peng and Zhang, 2002; Chitte and Dey, 2002). Therefore great attention is focused on production of more specific and less toxic fibrinolytic enzymes.

Staphylokinase

Staphylokinase is an ideal fibrin specific plasminogen activator that converts plasminogen to plasmin, which in turn attacks on the fibrin clots. SAK possess better fibrin specificity than t-PA and are capable of dissolving platelets-rich clots (Lijnen *et al.,* 1992; Collen *et al.,* 1993).

Structure

Staphylokinase is an extracellular protease of 136 amino acid and is a 15 kDa protein, produced during the late exponential phase by a lysogenic strain of *Staphylococcus aureus* (Hameed *et al.,* 2015). It is positively regulated by the "agr" gene regulator. It activates plasminogen to form plasmin, which digest fibrin clots. This disrupts the fibrin mesh-work which can often form to keep an infection localized. Staphylokinase interacts with plasminogen to form a 1:1 complex that exposes the active site of the plasminogen molecule. The plasmin Sak complex can be actively neutralized by α2-antiplasmin in plasma in the absence of fibrin, resulting in lysis. Staphylokinase also cleaves IgG and complement component C3b, inhibiting phagocytosis. It is classified under EC3.4.99.22. The full length of mature staphylokinase mRNA is 489bp. The first 27 amino acids codes for a signal peptide which is to cleave off in the mature SAK protein. The Shape of staphylokianse is elongated; it contains two folded domains which are of similar size (Rao *et al.,* 2013).

Sources : Clinical samples such as skin lesions, throat swabs, local wound, bovine milk, Staphylokinase Producers: *Staphylococcus aureus*, *Staphylococcus hominis* MSD1.

Production

Staphylokinase when produced from non-pathogenic samples make production process safe; reduce the chances of cross contamination, and cost of downstream processing. Attempts have been made to isolate and characterize staphylokinase producing pathogenic as well as non-pathogenic strain of milk, water and sewage was carried out and it was found that the isolated staphylokianse was about 15.5 kDa, consisting of 136 a.a single chains, which was able to digest the fibrin clots (Subathra Devi *et al.,* 2012). Staphylokinase producing *staphylococcus* spp. was successfully isolated from bovine milk as economical and safer clot buster as compared to EDTA and heparin (Rao *et al.,* 2013). Effect of different parameters and condition caused increase enzyme production such as the effect of UV mutation on enhancing the production of enzyme in the strain *S. aureus* VITSDVM7 was investigated and found to be effective with increase in the fold of purification (Srinivasan and Subathra, 2013; Mohansrinivasan and Subathra *et al.,* 2014).

Effectiveness of SAK needs to be improved as it lacks any affinity for fibrin and the ability to inhibit thrombin. Both of these functions can be combined with SAK by constructing translational fusions with fibrin-binding or anti-thrombin protein domains (Wnendt *et al.,* 1996). Studies were made to reduce the chance of pathogenicity by cloning staphylokinase gene from mutated *S. aureus* in *E. coli* (Hameed *et al.,* 2015). SAK is one of the bacterial proteins having good clot specificity, but it poses great risk in protein production as it is produced by pathogenic *S. aureus* (Wnendt *et al.,* 1996; Pulicherla *et al.,* 2011). Isolation of the mature staphylokinase gene from local wounds and cloning and expression from a salt inducible *E.coli* expression host found to be a potent way to decrease the effects of pathogenic strain (Wnendt *et al.,* 1996). The studies of the scale up production and purification of recombinant staphylokinase-based fused protein over expressed in *Escherichia coli,*

proved it to be a promising therapeutic candidate for thrombolytic disease (Zhong *et al.,* 2009).

Applications

1. Fibrin specific activator.
2. Role in anti-clotting functioning.
3. Dissolves plasminogen to inactive proenzyme plasmin, thus acting as clot busters.
4. Relatively inexpensive due its easy production and presence in environmental samples (Lack 1987).

Nattokinase

Nattokinase is an enzyme considered to be a promising remedy for thrombosis due to its presence in food and robust fibrinolytic activity. Nattokinase is an enzyme extracted and purified from a Japanese food called natto. Natto is made from fermented soyabeans. It is produced by fermentation by adding the bacterium *Bacillus natto* which is the preeminent nattokinase producer (Haritha and Meena, 2011). Natto has a very strong, pungent flavor with cheesy smell and is a slimy paste. Nattokinase is also reported to contain vitamin K2 (mena quinone). NK was first isolated and marketed under the name of NSK-SD in 1998 by Japan Bioscience Laboratory (Tai and Sweet, 2006; Ku *et al.,* 2009). The action of nattokinase on the cleavage of fibrinogen in the plasma is remarkably prolonged in plasma sample draw 3 to 5 hours after administration of the enzyme. It digests fibrin directly or indirectly. It reduces blood pressure in human and is reported to lower both systolic and diastolic blood pressure (Maruyama and Sumi, 2015). Researchers suggest that nattokinase may promote normal blood pressure, reduce whole blood viscosity and increase circulation being an effective supplement to support cardiovascular disease (Kim *et al.,* 1996).

Structure

It is a serine protease of the subtilisin family with 275 amino acid residues and molecular weight of 27,728 Dalton having potent fibrinolytic activity (Mohansrinivasan and Subathra, 2014). Researches by DNA sequencing showed that the homology was 99.5 and 99.3% like subtilisin E and amylosaccharides respectively (Tai and Sweet, 2006; Ku *et al.,* 2009). The enzyme catalyzes the cleavage of protein to polypeptides. It can withstand temperatures of up to 50°C and repeated freezing and thawing, but is inactive in acidic conditions (Fujita *et al.,* 1993; Wang *et al.,* 2009).

Sources : Fermented food such as natto, soil samples, agricultural wastes.

Nattokinase producers : *Bacillus natto, Bacillus subtilis* and *Bacillus amyloliquefaciens*

Production

The large scale and easy production has made it commercially important. Different methods have been employed to optimize nattokinase medium component and production by using various statistical methods. From the study of optimized medium component and condition for the production of nattokinase, it was found that wheat bran was the best source for the production (Wang *et al.,* 2009). The optimization of nutritional conditions for nattokinase production by Bacillus natto NLSSE using statistical methods was done. Fractional Factorial Design was applied to elucidate the key ingredients in the media and the result indicated that the soy peptone, yeast extract and calcium chloride have a significant effect on nattokinase production (Liu *et al.,* 2005). Researchers have employed Response Surface Methodology and Central Composite Rotary Design to optimize fermentation medium for the production of nattokinase by *Bacillus subtilis* at pH 7.5. The optimized medium containing (%)

glucose: 1, Peptone: 5.5, MgSO4: 0.2 and $CaCl_2$: 0.5 resulted in 2-fold increased level of nattokinase production as compared to the initial level after 10 hr of fermentation (Deepak *et al.,* 2008). Reports were found on fibrinolytic enzyme producing strain from *Bacillus* subtilis LD-8547 from douche; a traditional Chinese soybean fermented food and improved the strain by mutating it with U.V, NTG and γ- radiation. From the results obtained it was concluded that mutagenesis for breeding was a useful and effective method for strain screening of high production (Wang *et al.,* 2011). When about 6,400 FU was administered orally for 4 consecutive days to human volunteers with high blood pressure, it was noted that there was a decrease of systolic as well as diastolic blood pressure (Maruyama and Sumi, 2015).

Applications

1. Clot buster
2. Blood thinner
3. Prevent from acute cerebellar hemorrhage
4. Reduce blood pressure
5. Reduce poor circulation
6. Oral administration
7. Anti-hypertension
8. Digestive capability (Yokota *et al.,* 1996 ; Mukesh et al., 2013).

Streptokinase

The extracellular enzyme streptokinase is produced by various strains of β-haemolytic *Strepto cocci.* Streptokinase producing Streptococci were first identified in 1874 by Billroth in exudates of infected wounds. The complexes of streptokinase with human plasminogen can hydrolytically activate other unbound plasminogen by activating through bond cleavage to produce plasmin (Mundada *et al.,* 2003). It is marketed in Chile as streptase by Alpes Selection. Streptokinase is now included in the World Health Organization Model list of Essential Medicines.

Structure

Streptokinase is a group of extracellular proteins and is a plasminogen activator 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 degrades fibrin clot through its specific lysine binding site. The enzyme is a single polypeptide that exerts its fibrinolytic action indirectly by activating the circulatory plasminogen. Streptokinase produced by different groups of Streptococci differs considerably in structure (Malke and Feretti, 1993). Streptokinase consists of multiple structural domains (i.e. α, β, γ domains) with different associated functional properties. Scanning calorimetric analysis suggests that the protein is composed of 2 distinct domains (Welfle *et al.,* 1992). The N-terminal domain has been found to complement the low plasminogen activation ability of the 60-414 amino acid residue domain of protein (Nihalani *et al.,* 1998). Plasminogen of human, chimpanzee, monkey, cat, dog and rabbit are the only known protein substrate for streptokinase (Castellino *et al.,* 1981). It is widely used as a thrombolytic agent in the treatment of acute myocardial infarction, including coronary thrombosis (Kim *et al.,* 2000).

Sources : Throat swab, blood samples, tonsilitis

Streptokinase producers : *Bacillus licheniformis, Streptococcus pyogens, Streptococcus equisimilis*

Production

Due to its expensive production cost different methods are employed to increase the rate of production and reduce it pathogenicity. Native streptokinase is useful for cost-effective thrombolytic theraphy in clinical practice, but not risk free. Large quantities of streptokinase can be produced inexpensively via bacterial

fermentation. Cloning of the streptokinase gene in non-pathogenic microorganisms has enabled production of recombinant streptokinase that eliminated any risk. Various chemical modifications of streptokinase have been used for extending its half-life in circulation, improving plasminogen activation and eliminating or reducing immunogenicity. Investigation of the production of streptokinase by β-haemolytic Streptococci isolated from throat of infected patients was performed. The strain was also improved by u.v mutagenesis. The study revealed that the mutant strain produced more enzymes as compared to wild type. It was also concluded that U.V mutation is the best way to enhance the production of fibrinolytic enzyme (Madhuri *et al.,* 2011). The effect of mutation on the production of streptokinase by β-haemolytic Streptococci was analysed. The organism was identified as *Streptococcus equisimilis*. The strain was mutated with U.V and N-methyl-N'-nitro-N-Nitroso Guanidine (NTG). Both the mutagen was found to increase the productivity of SK production as compared to wild type (Abdelghani *et al.,* 2005). Studies were made on the role of streptokinase as a virulence determinant of *Streptococcus* pyogens in invasive pathogenesis and develop useful strategies that disrupt streptokinase mediated plasminogen activation and could be employed to treat severe invasive *S. pyogensin* fections (McArthur *et al.,* 2012).

Applications

1. Intravenously injected
2. Fast action
3. Prevent post-operative adhesion
4. Prevent thromboembolic blockages (Dubey *et al.,* 2011)

Side Effects of Streptokinase

1. Bleeding due to activation of circulating plasminogen.
2. Antigenicity and high titer antibodies develop 1 to 2 weeks after use, precluding retreatment until the titer declines.
3. Allergic reactions like rashes fever, hypotension (Battershill, 1994).

Conclusion

The present review states about the action, sources, production of fibrinolytic enzymes and its application as a thrombolytic agent. Fibrinolytic enzymes are too costly and also needs large scale production by some alternative methods with high purity. So, isolation, production, purification, assay and characteri zation of fibrinolytic enzymes from bacterial sources are very effective and useful.

References

Abdelghani, T.T.A., Kunamneni, A. and Ellaiah, P. 2005. Isolation and mutagenesis of streptokinase producing bacteria. *Am. J. Immunol.,* 1(4): 125 - 129.

Anjana, S., Arunima, S. and Shivlata, L. 2015. Optimization of medium components for enhanced production of extracellular fibrinolytic protease from citro bacter braakii. *International Journal of current microbiology and Applied Sciences*., 4(7) : 248 - 259.

Battershill, PE., Benfield, P. and Goa, K.L. 1994. Streptokinase: A review of its pharmacology and therapeutic efficacy in acute myocardial infraction in older patients. *Drugs Aging.,* 4 : 63 - 86.

Castellino, F.J. 1981. *Trends Biochem. Sci. Pers .* 4 : 1 - 5.

Chitte, R R. and Dey, S. 2000. Potent fibrinolytic enzyme from a thermophilic *Streptomyces megaspores* strain sd5. *Let. Appl. Microbial.,* 31(6): 405 - 410.

Chitte, R.R. 2013. Proteases in health disease. Springer, New York.

Chitte, R.R. and Dey, S. 2002. Purification of a fibrinolytic enzyme by thermophilic streptomyces species. *World J. Microbiol. Biotechnol.,* 18(4) : 289 - 294.

Collen, D. and Lijnen, H.R. 1994. Staphylokianse, A fibrin specific plasminogen activator with therapeutic potential. *Blood.,* 84 (3) : 680-686.

Collen, D., F. De Cock, and Stassen, J.M. 1993. Comparative immunogenicity and thrombolytic properties toward arterial and venous thrombi of streptokinase and recombinant staphylokinase in Baboons. *Circulation.,* 87 : 996 – 1006.

Deepak, V., Kalishwaralal, K., Ramkumar pandian, S., Babu, S.V. Senthilkumar, SR. and Sangili yandi, G. 2008. Optimization of media composition for nattokinase production by *bacillus subtilis* using response surface methodology. *Biores. Technology.,* 99 : 8170 - 8174.

Dubey, R.J., Kumar, D., Agrawala, T., Char and Pusp, P. 2011. Isolation, production, purification, assay and characterization of fibrinolytic enzymes (nattokianse, streptokinase and urokinase) from bacterial sources. *African Journal of Biotechnology.,* 10(8): 1409 - 1420.

Dubis, J. and Witkiewicz, W. 2010. The role of thrombin-activatable fibrinolysis inhibitor in the pathophysiology of haemostasis. *Adv. Clin. Exp. Med.,* 19(3) : 379 - 387.

Essam, F., Juamily, A. and Bushra, H. 2012. Optimization conditions of production fibrinolytic enzyme from *Bacillus licheniformis* b4 isolate. *British Journal of Pharmacology and Toxicology.,* 3(6): 289-295.

Fujita, M., Nomura, K., Hong, K., Ito, Y., Asada, A. and Nishimuro, S. 1993. Purification and characterization of a strong fibrinolytic enzyme (nattokinase) in the vegetable cheese natto, a popular soybean fermented food in Japan. *Biochem. Biophys. Res. Commun.,* 197 : 1340 - 1347.

Gerheim, E.B. 1948. *Staphylococcal* Coagulation and Fibrinolysis. *Nature.,* 162 - 732.

Gull-E-Faran, A.Z., Muhammad, S., Muhammad, and sajid, A. 2015. Improved streptokinase production; uv irradiation of *streptococcus equisimilis*. *The Professional Medical Journal.,* 22(5) : 656 - 663.

Hameed, J., Hamzah, A. and Dellol, R. 2015. Optimum conditions of staphylokianse production cloned in *E. coli* jm109. *Int. J. Curr. Microbiol. App. Sci.,* 4(12) : 10 - 19.

Haritha, M. and Meena, V. 2011. Nattokinase : A review on fibrinolytic enzyme. *International Journal of chemical, environment and Pharmaceutical research*., 2(1) : 61-66.

Kim, D.M., Lee, S.J., Kim, I.C., Kim, S.T. and Byun, S.M. 2000. Asp14-His48 region of Streptokinase is important in binding to substrate plasminogen. *Thromb. Res.,* 99 : 93 - 98.

Kim, W., Choi, K., Kim, Y., Park, H., Choi, J. and Leey, H., Kwon, I. and Lee, S. 1996. Purification and characterization of a fibrinolytic enzyme produced from *Bacillus* sp. strain ck11-4 screened from chungkookjang. *Appl. Environ. Microbiol.,* 62(7) : 1488 - 2482.

Koide, A., Suzuki, S. and Kobayshi, S. 1982. Preparation of polyethylene glycol-modified streptokinase with disappearance of binding ability towards antiserum and retention of activity. *FEBS Lett.,* 143 : 73 - 76.

Ku, TW., Tsai, RL. and Pan, TM. 2009. A simple and cost saving approach to optimize the production of subtisilin NAT by submerged

cultivation of *Bacillus subtilis natto*. *J. Agric. Food. Chem.,* 57(1) : 292 - 296.

Lack, C.H. 1987. Staphylokiansean activator of plasma protease. *Nature.,* 161 : 559 - 560.

Lee, J., Park, S., Choi, W.A., Lee, K.H., Jeong, Y.K., Kong, I.S. and Park, S. 1999. Production of a fibrinolytic enzyme in bioreactor culture by *Bacillus subtilis* bk-17. *J. Microboil. Biotechnol.,* 9(4) : 443 - 449.

Lijnen, H.R., Van Hoef, B. Vanderbossche, L. and Collen, D. 1992. Biochemical properties of natural and recombinant staphylokinase. *Fibrinolysis.,* 6 : 214 - 225.

Liu, J., Chang, T., Xing, J., Zhiya, M. and Liu, H. 2005. Optimization of nutritional conditions for nattokianse production from *Bacillus natto* NL SSE using statistical experimental methods. *Process Bio- chemistry.,* 40(8) : 2757 - 2762.

Madhuri, D., Madhuri, M. and Neha, S. 2011. Studies on isolation, screening and strain improvement of streptokinase producing-hemolytic *Streptococci*. *World Journal of Nuclear Science and Technology.,* 1(1): 7 - 11.

Malke, H. and Feretti, J.J. 1993. Streptokinase : cloning, expression and excretion by *Escherichia coli. Proc. Natl. Acad. Sci.* USA. 81 : 355 - 3561.

Maruyama, M. and Sumi, H. 2015. Effect of natto diet on blood pressure*. Basic and Clinical aspects of Japanese Traditional Food Natto.,* 2 : 1 - 3.

McArthur, J.D., Cook, S.M., Venturini, C. and Walker, M. 2012. The role of a Streptokinase as a virulent determinant of *Streptococcus pyogens*- potential for therapeutic targeting. *Current Drug Targets*., 13(3) : 297 - 307.

Mohansrinivasan, V. and Subathra, C. 2014. Production of fibrinolytic staphylokianse from UV mutated *Staphylococcus aureus* vitsdvm7. *International Journal Of Chem-tech Research*., 6(8) : 4007 - 4014.

Mukesh, D.J., Rakshita, R., AnnuVidhya, M., Jennifer, S., Sandip, P., Ravi, M. and Kalai chalvan, P.T. 2014. Production, optimization and characterization of fibrinolytic enzyme by *Bacillus subtilis* RJAS19. *Pakistan Journal of Biological Sciences.,* 17 (4) : 529 – 534.

Mundada, L.V., Prorok, M., Deford, M.E., Figuera, M., Castellino, F.J. and Fay, W.P. 2003. Structure-function analysis of the Streptokinase amino Terminus (Residues 1-59). *The Journal of Biological Chemistry.,* 273 (5): 3110 - 6.

Nascimento., Wellingta Cristina Almeida do and Martins, Meire Lelis Leal. 2004. Production and properties of an extracellular protease from thermophilic *Bacillus* sp. *Brazilian Journal of Microbiology.,* 35(1-2) : 91 - 96.

Nihalani, D., Kumarm, K., Rajagopal and Sahni, G. 1998. Role of amino-terminal region of streptokinase in the generation of a fully functional plasminogen activator complex probed with synthetic peptides. *Protein Sci.,* 7 : 637 - 648.

Peng, Y. and Zhang, Y.Z. 2002. Cloning and expression in E.Coli of coding sequence of the fibrinolytic enzyme mature peptide from *Bacillus amyloliquefaciens* DC-4. *Chin. J. Appl. Environ. Biol.,* 8 : 285 - 289.

Pulicherla, K., Gadupudi, G.S., Rekha, V.P.B., Seetharam, K. Anmol, K. and Rao, S. 2011. Isolation, cloning and expression of mature staphylokinase from lysogenic *Staphylococcus aureus* collected from a local wound sample in a salt inducible *E. coli* expression

host. *Int. J. of Advanced Science and Technol.,* pp. 30.

Rajagopalan, S., Gonias, S.L. and Pizzo, S.V. 1985. A Non-Antigenic covalent Streptokinase Polyethylene glycol Complex with Plasminogen activator function. *J. Clin. Invest.,* 75 : 413 - 419.

Raju, E. and Divakar, G. 2013. Screening and isolation of fibrinolytic protease producing mesophilic bacteria from slaughter houses in Bangalore. *International Journal of Pharmaceutical Sciences And Research.,* 4(9): 3625 - 3629.

Rana, El-baz., R. Dina, B. Rasha, and Ramadan, H. 2016. Virulence factors profile of *Staphylococcus aureus* isolated from different clinical sources. *N. Egypt. J. Microbiol.,* 43 : 126 -144.

Rao, S., Kumar, A., Peravali, J.B., Ram, K.S. and Pulicherla, K. K. 2013. Staphylokinase: a boon in medical sciences. *Mintage journal of pharmaceutical and medical sciences.,* 2(2): 28 - 34.

Srinivasan, S. and Subathra, C. 2013. Screening for staphylokinase producing *Staphylococcus* spp. from bovine milk sample. *International journal of pharmacy and Pharmaceutical Sciences.,* 5(2) : 601 – 604.

Subathra Devi, C., Dipti, S., Vaibhavi, S. and Mohanasrinivasan, V. 2012. Screening For Staphylokianse producing *Staphylococcus* Spp. from different Environmental samples. *Asian Journal of Pharmaceutical and Clinical Research*., 5 : 125 – 128.

Sumi, H., Hamada, H., Tsushima, H., Mihara, H. and Muraki, H. 1987. A novel fibrinolytic enzyme (Nattokinase) in the vegetable cheese natto; a typical and popular Soyabean food in the Japanese diet. *Experientia.,* 43(10) : 1110 - 1111.

Suzuki, Y., Kondo, K., Ichise, H., Tsukamoto, Y., Urano, T. and Umemura, K. 2003. Dietary supplementation with fermented soybeans suppresses Intimal thickening. *Nutrition.,* 19 : 261.

Tai, M.W. and Sweet, B.V. 2006. Nattokinase for prevention of thrombosis. *Am. J. Health. Syst. Pharm.,* 63(12):1121-1123.

Venkata, E. and Divakar, G. 2014. An overview on microbial fibrinolytic proteases. *Int. J. Pharm. Sci. Res.,* 5(3): 643 - 56.

Voet, D. and Voet, J.G. 1990. Biochemistry. Wiley, New York. pp. 87-95.

Wang, C., DU, M. and Zheng, D. 2009. Purification and characterization of nattokinase from *Bacillus subtilis natto* B-12*. J. Agric Food Chem.* 57(20) : 9722 – 9.

Wang, S.L., Wu, Y.Y. and Liang, T.W. 2011. Purification and biochemical characterization of a nattokinaseby conversion of shrimp shell with *Bacillus subtilis* tku007. New Biotechnology., 28 : 196 - 202.

Welfle, K., Pfeil, W., Misselwitz, and Welfle, H. 1992. Conformational properties of Streptokinase differential scanning Calori metric investigations. *Int. J. Biol. Macromol.,* 14 : 19 - 22.

WHO. 2015. Model List of Essential Medicines.

Wnendt, S., Janocha, E., Schneider, J. and Steffens, G.J. 1996. construction and structure-activity reationships of chimeric prouro kinase derivatives with intrinsic thrombin - inhibitory potential. *Protein. Eng.,* 9 : 213 - 223.

Yokota, T., Hattori, T., Ohishi, K., Hasegawa and Wantanabe, K. 1996. The effect of anti-oxidant containing fraction from fermented soyabean food on atherosclerosis development in cholesterol-fed rabbits. *LWT-Food Sci.,* 29 : 751 - 755.

Zakaria, Z., Salleh, M. and Rashid, N. 2015. Screening and identification of fibrinolytic bacteria from Malaysian fermented sea-foods products. *Journal of Applied Pharmaceutical Science*., 5(10) : 022 - 031.

Zhong, G., Aiping, Y.U., Bingxing, S.H.I., Yang, L.I.U., and Chutse, WU. 2009. Pilot-scale production and purification of staphylo-kianse-based fusion protein over expressed in *Escherichia coli.*

http://i47.tinypic.com/29q1wdl.png.

http://www.cslbehring.com/

https://en.m.wikipedia.org/wiki/Nattokinase.

http://www.who.int/medicines/publications/essentialmedicines/en.

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