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SCREENING OF STAPHYLOKINASE PRODUCING *STAPHYLOCOCCUS SPP*. FROM DIFFERENT SOURCES

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ABSTRACT
Thrombolytic agent plays an important role in the treatment of cardiovascular diseases. The search for an efficient thrombolytic agent is a continuous process. SAK is a bacterial kinase produced by certain strains of *Staphylococcal spp.* which activates plasminogen to form plasmin and digest fibrin clots. The recent studies on staphyokinase prove it to be a promising thrombolytic agent. The present study focuses on screening of staphylokinase producing *Staphylococcus spp*. from different environmental, medical and skin flora. The screening of isolates was carried on nutrient agar, mannitol salt agar, blood agar, and casein agar plates. Isolates were selected on the basis of gram staining, and growth characteristics showed on nutrient agar, blood agar, gelatin agar and casein agar plates respectively. The selected isolates were white, cream, golden, or yellow color appearing as gram positive cocci in clusters or singly showing β-haemolytic activity on blood agar plates, and proteolytic activity on casein agar plates. Based on the result of screening, out of 28 isolates, 10 probably which may be potent staphylokinase producing organisms were selected for Heated Plasma Agar Assay. The future study will focus on characterization, production and purification of economical and safe clot buster agent.

KEY WORDS: Thrombolytic activity, Staphylokinase, Plasminogen, Mannitol agar, Blood agar, β-Haemolytic, Heated Plasma Agar.

INTRODUCTION

Cardiovascular diseases are one the most important cause of mortality worldwide. Intravascular thrombosis due to the accumulation of blood clot in the blood vessels is the main cause for cardiovascular diseases. Fibrinolytic disorders contribute to thrombosis, artherosclerosis, endometriosis, complication in pregnancy and development of neoplastic disease (Dubis, 2010). There are many research available involving screenings, and isolation of micro-organism producing enzyme with high fibrinolytic activity, because most of the commercial available enzymes have complication such as, bleeding, allergic, expensive, re-occulusion *etc*. (chitte, 2013). Fibrinolytic enzyme is well known as a sub-class of protease which has the ability to degrade protease. Fibrinolytic enzymes convert plasminogen to plasmin and lyse clots by breaking down the fibrin contained in the clot. Novel fibrinolytic enzymes derived from microbial source are useful for thrombolytic therapy. Fibrinolytic enzymes have been isolated from different sources and have been proved as effective thrombolytic agents. Fibrinolytic enzymes have been reported from various bacterial species such as *Bacillus, Staphylococcus, Aspergillus*, *Penicillium,* Mushrooms *etc.* There are different fibrinolytic enzymes available commercially such as Staphylokinase, Streptokinase, Nattokinase, t-PA, Thrombokinase, Urokinase.

Staphylokinase is an ideal fibrin specific plasminogen activator, which converts plasminogen to plasmin which in turn attacks on the fibrin clots. Staphylokinase is 136 aminoacid extracellular proteins produced during the late exponential phase by lysogenic strain of *Staphylococcus aureus* (Hameed *et al*., 2015). SAK is one of the bacterial proteins having good clot specificity but it pose great risk in protein production as it is produced by pathogenic *S. aureus* (Pulicherla *et al*., 2011). Staphylokinase when produced from non-pathogenic samples make production process safe; reduce the chances of cross contamination, and cost of downstream processing (Jin *et al*., 2004).

MATERIALS AND METHODS

Sample Collection

Samples were collected from skin swab, slaughter house, hospitals waste, environmental samples like soil and water. All samples were collected and brought under aseptic conditions to laboratory and stored at 4°C.

Isolation and Morphological Characterization

All samples were first screened on Nutrient Agar Plate to obtain desired isolates and were incubated for 24 hours at 37°C. The staphylococcal colonies were selected on the basis of colonial, morphological characteristics, motility, catalase test and Gram's reaction. The *Staphylococcus*was screened using selective media – Mannitol Salt Agar Plate and β-Haemolytic properties were studied on Blood Agar Plate. The colonies showing β-haemolysis were selected for further use and preserved at 4°C on nutrient agar slant.

Screening for Staphylokinaseproducing *Staphylococcus*

The isolates obtained were screened on Mannitol Salt Agar Plate, Blood Agar plate, Casein Agar Plate and Heated Plasma Agar Plate to check its mannitol fermentation, haemolytic, proteolytic and plasmolytic activity.

Mannitol Fermentation

The organisms were checked for their ability to ferment mannitol sugar. Mannitol salt agar plate contains 7.5% salt that allows the growth of *Staphylococcus spp*. The isolates

were streaked on to the plates and incubated for 24 hours at 37°C.

β-Haemolysis Assay

Blood agar plates were prepared by mixing fresh blood obtained from healthy volunteer to nutrient agar. The plates were allowed to solidify and the isolates were placed on to the plate. The plates were incubated for 24 hours at 37°C.

Casein Hydrolysis Assay

Casein hydrolysis assay was done by mixing non-fat milk and agar. The plates were allowed to solidify and the isolates were streaked on to the plate. The plates were then incubated for 24 hours at 37°C.

Heated Plasma Agar Assay

Heated plasma agar assay is one the method used to check the plasmolytic activity of staphylococcus isolates. Plasma for the assay was obtained by collecting 10ml. of blood from healthy volunteer in an anticoagulant. The blood was centrifuged at 15000 R.P.M for 15 minutes. The plasma obtained was mixed with nutrient agar in the ratio of 3:1, and the plates were prepared. The isolates were then streaked on to the plate and incubated for 24 hours at 37°C.

RESULTS AND DISCUSSION

Isolation and Morphological Characteristics

Out of 102 samples, 28 isolates were selected based on the morphological and colonial characteristics. The selected isolates were found to be white, orange or yellow colonies and Gram positive cocci occurring singly or in clusters during Gram staining. These same results were observed in the work of (Subathra Devi *et al*., 2012). The isolates obtained were non-motile and catalase positive. The isolates were then used for further work.

Mannitol Fermentation

The colonies which were able to ferment mannitol and showed yellow color were probably assumed as *Staphylococcus spp.* (fig. 1). S-34(1), 42, 44, 56, 90, 90(1), 91, 94, 95, 96, 97 were mannitol fermentor, whereas the remaining isolates couldn't ferment mannitol. In recent studies it has been proved that certain strains of *Staphlyococcus* doesn't ferment mannitol, so the mannitol non-fermentor were further confirmed by heated plasma agar assay.

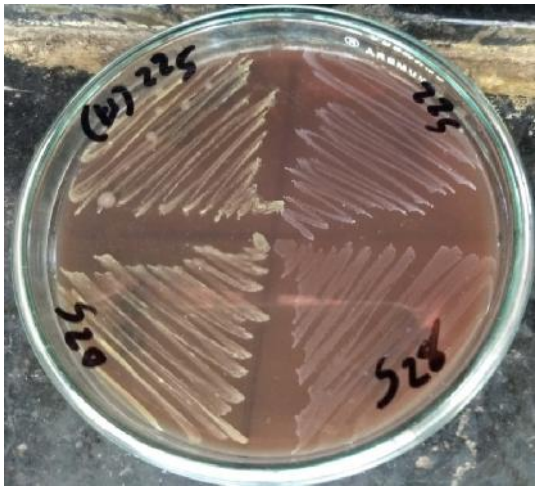

FIGURE 1: Results given by isolates on Mannitol Salt agar showing Mannitol Fermentor& Non- Fermentor

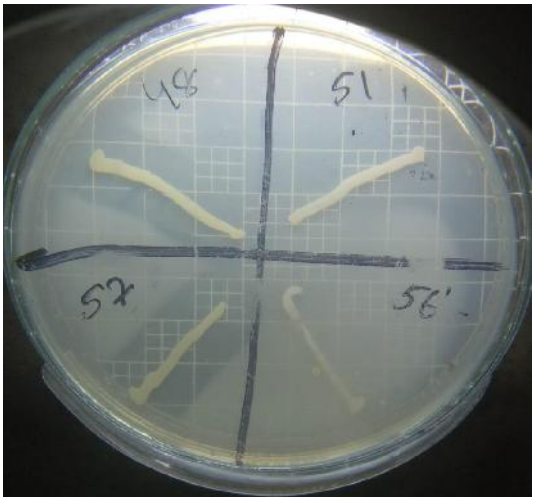

FIGURE 2: Results given by isolates on Casein Agar Plate

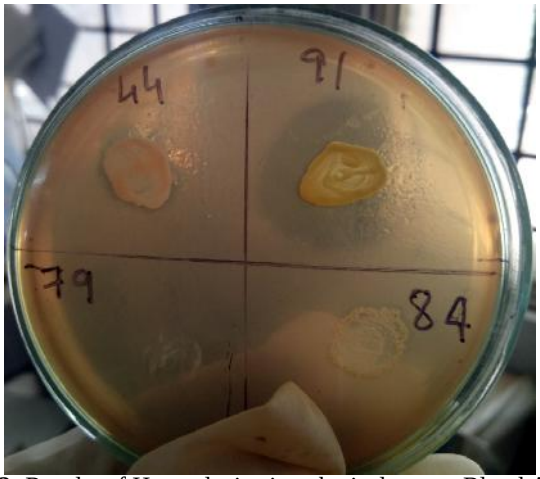

FIGURE 3: Results of Haemolysis given by isolates on Blood Agar Plate

Casein Hydrolysis Assay

The isolates showed clear hollow zone around the colonies (Fig. 2). This indicates that the isolates produce proteases that may indicate the production of staphylokinase. The result of casein hydrolysis is similar to Shagufta *et al*., 2014 and Subathra Devi *et al*., 2012. The current study shows that the isolates selected where all showing casein hydrolysis test positive.

β-Haemolysis Assay

The isolates obtained on the blood agar plate showed β-haemolysis (Fig 3). The zone size of hydrolysis was measured and it was found that out of 28 isolates, 10 isolates showed maximum zone of hydrolysis (Table 1). The highest zone measured was 22 mm. Subathra Devi C, *et al*., 2012 also checked for haemolysis and showed the pattern of haemolysis on blood agar plate.

TABLE 1: Result of selected isolates on Blood Agar Plate

Isolates	β-haemolysis(in mm)	Isolates	β-haemolysis(in mm)
S-34(1)	14±0.2	S-86	10±0.5
S-34(2)	11±0.5	S-86(1)	9±1.0
S-34(3)	11±1.0	S-86(2)	8±0.3
S-42	18±0.8	S-87	11±0.6
S-44	13±0.5	S-90	20±0.2
S-48	10±0.2	S-90(1)	12±0.5
S-56	16±0.5	S-91	22±0.8
S-72	9±1.0	S-92	10±0.6
S-72(1)	9±1.2	S-93	11±0.5
S-74	10±0.5	S-94	18±0.2
S-77	8±0.7	S-95	22±0.6
S-79	10±0.3	S-96	22±0.5
S-84	12±0.2	S-97	20±1.0
S-85	12±0.5	S-98	12±1.5

Heated Plasma Agar Assay

Out of 10 isolates, 5 isolates showed positive plasmolytic activity which indicates that the 5 isolates obtained were staphylokinase producing *Staphylococcus spp*. (Fig 4). The same result was obtained by Subathra Devi *et al*., 2012 and Shagufta *et al*., 2014. The current study shows the production of staphylokinase from the selected isolates (Table 2).

FIGURE 4: Results Given by Isolates on Heated Plasma Agar Plate

TABLE 2: Heated Plasma Agar Assay for the Selected Isolates

Isolates	Halo Formation
S-56	Negative
S-44	Negative
S-90	Positive
S-95	Positive
S-97	Negative
S-96	Positive
S-91	Negative
S-42	Negative
S-34	Positive
S-94	Positive

CONCLUSION

Myocardial infarction has tremendously increased in recent years leading to heart attack and strokes. Fibrinolytic enzymes have been found potent thrombolytic agent. It has been isolated from different organisms with an aim to increase the production and decrease the cost of existing thromobolytic agents. In the present study the focus is made on staphylokinase enzyme from staphylococcus spp. Obtained from varoius environmental samples. All 28 isolates were screened for positive mannitol fermentation, casein hydrolysis, β-Haemolysis and heated plasma agar assay. From the 28 isolates, 10 isolates were selected based on the result on casein agar, blood agar and heated plasma agar assay. Out of 10 isolates, all isolates showed haemolytic and proteolytic activity, and 5 isolates showed plasmolytic activity. The study reveals that the selected isolates can be promisingstaphylokinase producers. They can be effectively used in future for cheap and efficient production of thrombolytic agent.

REFERENCES

Adam, N.O., Alawad, A.M., Enan, K.A., Mustafa, M.O. & Ibrahim, H.M. (2016) Screening and Molecular Characterization of Staphylokinase Producing *Staphylococcus spp* Isolated from Bovine Milk. *International Journal of Advances in Pharmacy, Biology and Chemistry*, 5(3) 2277-4688.

Bhardwaj, S., Angayarkanni, J., Bhattacharya, S., Das, A., &Palaniswamy, M. (2013) Isolation, Screening and Characterization of β-Haemolytic Streptococci with Potential of Streptokinase Production. *International Research Journal of Biological sciences*, 2(4), 63-66.

Chitte, R.R. (2013) Proteases in Health Disease. *Springer*, New York.

Collen, D. (1998) Staphylokinase: A Potent, Uniquely Fibrin-Selective Thrombolytic Agent. *Nature medicine*, *4*(3), 279-284.

Collen, D., De Cock, F. & Stassen, J.M. (1993) Comparative Immunogenicity and Thrombolytic Properties toward Arterial and Venous Thrombi of Streptokinase and Recombinant Staphylokinase in Baboons.*Circulation*, *87*(3), 996-1006.

Collen, D., Nossel, H.L. & Vogel, H.J. (1982) Regulation of Fibrinolysis: Plasminogen Activator as A Thrombolytic Agent. In *Pathobiology of the Endothelial Cell*. Academic Press, New York-London, 183-189.

Das, G. & Prasad, M.P. (2010) Isolation, Purification & Mass Production of Protease Enzyme from *Bacillus subtilis*. *Int. Res. J. Microbiol.*, *1*(2), 26-31.

Dubis, J. & Witkiewicz, W. (2010) The Role of Thrombin-Activatable Fibrinolysis Inhibitor in the Pathophysiology of Haemostasis. *Adv Clin Exp Med*, 19(3):379-387.

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

Jasim, H.M., Dellol, R.A. & Hamzah, A.S. (2015) Optimum Conditions of Staphylokinase Production Cloned in E. coli Jm109 (De3). *Int. J. Curr. Microbiol. Appl. Sci*, 4(12), 10-19.

Jin, T., Bokarewa, M., Foster, T., Mitchell, J., Higgins, J., & Tarkowski, A. (2004) *Staphylococcus aureus* resists human Defensins by Production of Staphylokinase, A Novel Bacterial Evasion Mechanism. *The Journal of Immunology*, 172(2), 1169-1176.

Lack, C.H. (1987) Staphylokinase an Activator of Plasma Protease. *Nature*, 161:559-560.

Lijnen, H.R., Van Hoef, B., De Cock, F., Okada, K., Ueshima, S., Matsuo, O., & Collen, D. (1991) On the Mechanism of Fibrin-Specific Plasminogen Activation by Staphylokinase. *Journal of Biological Chemistry*, *266*(18), 11826-11832.

Mohana, S.V., Subathra Devi, C., Dhanmoni, K., Vaishnavi, B., Jemimah, N.S. & Kaustuvmani, P. (2015) Production and Purification of Staphylokinase from *Staphylococcus hominis* MSD1 Isolated from Kadi: A Traditional Indian Fermented Food. *International Journal of Pharm Tech Research*, *8*(6), 265-272.

Pulicherla, K.K., Gadupudi, G.S., Rekha, V.P.B., Seetharam, K., Kumar, A. & Rao, K.R.S.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. *International Journal of Advanced Science and Technology*, *30*, 35-42.

Raju, E.V.N. & 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.

Rana, El-baz., Dina, R. Rasha, B. and Ramadan, H., (2016) Virulence Factors Profile Of Staphylococcus Aureus Isolated From Different Clinical Sources. *N. Egypt. J. Microbiol*., 43.

Rao, S., Kumar, A., Peravali, J.B., Ram, K.S., & Pulicherla, K.K. (2013) Staphylokinase: A Boon in Medical Sciences. *Mintage Journal of Pharmaceutical and Medical Sciences*, 2 (2)28-34.

Reddy, Y.G.P., Prakash, Rajendran, & Anandakumar, S. (2014) Isolation, Cloning and Expression of Recombinant Staphylokinase Gene against Thrombosis. *J Pharm PharmSci*, *6*(4), 266-270.

Shagufta Naseer, B., Ravi, M., Subhashchandra, M.G. & YMJ. (2014) Screening of Staphylokinase producing *Staphylococcus aureus* from Clinical Samples. *International Journal of Research in Biological Sciences*, *4*(2), 46-48.

Srinivasan, V.M., Devi, C.S., Saha, A., Mondal, M., Dhar, S., Suganthi, V. & Selvarajan, E. (2013) Screening for Staphylokinase producing *Staphylococcus spp*. from Bovine Milk Sample. *Journal of Pharmacy and Pharmaceutical Sciences*, *5*(2), 601-604.

Subathra, C., Sinha, D., Sharma, V. & Srinivasan, V.M. (2012) Screeining for Staphylokianse Producing *Staphylococcus* Spp. from Different Environmental Samples.*Asian Journal of Pharmaceutical and Clinical Research*. 5(4), 0974-2441.

Vesterberg, K. & Vesterberg, O. (1972) Studies on Staphylokinase.*Journal of medical microbiology*, *5*(4), 441-450.

Więckowska-Szakiel, Marzena., Sadowska, Beata, & Różalska, B. (2007) Staphylokinase Production by Clinical *Staphylococcus aureus* Strains.*Polish journal of microbiology*, *56*(2), 97-102.