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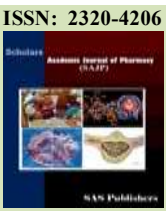

Short Communication

Screening and isolation of fibrinolytic protease producing bacteria from various regions in Bangalore

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Abstract: This work has been undertaken for the screening and isolation of fibrinolytic protease producing strains of Bacteria were carried out from Ten soil samples, collected from various regions of Bangalore and used to screen for fibrinolytic protease production by using fibrin plate assay. In the present study, an attempt was made to isolate efficient fibrinolytic protease producing bacteria from diverse environmental samples. Different isolates were screened for possessing the ability to produce fibrinolytic protease. About 6 bacterial isolates were found to be promising to produce fibrinolytic protease. The organisms were tested for various biochemical tests, which leads to their identification as *Bacillus licheniformis, Bacillus cerus,* and *Staphylococcus aureus*
Keywords : Fibrinolytic protease, Bangalore, fibrin plate, *Bacillus licheniformis, Bacillus cerus, Staphylococcus aureus*

INTRODUCTION

Enzymes are delicate protein molecules necessary for life. fibrinolytic Proteases are the single class of enzymes which play an important part in the metabolism of almost all organisms (Plants, Animals, Fingi, Bacteria and Viruses)**[1].**Investigation of fibrinolytic proteases is a central issue in enzymology due to their wide applications in Clinical ,Pharmaceutical,Food, and Bioremediation process. Among the various proteases, bacterial extracellular proteases are the most significant, compared with animal,Plants,viruses and fungal extracellular proteases. Extracellular proteases produced by Bacillus and cocci species are of main interest from a biotechnological perspective, and are not only in scientific fields of protein chemistry and protein engineering but also in applied fields such as foods and pharmaceutical industries. These fibrinolytic proteases account for 60% of the total worldwide production of enzymes**[2]**. The genus Bacillus and cocci contains a number of industrially important species and approximately half of the present commercial production of bulk enzymes derives from the srtains of *Bacillus* and *cocci*. fibrinolytic proteases belongs to the class hydrolase which are able to hydrolyse insoluble fibrin more efficient than other proteases and their action are very specific, i.e., they acts only on fibrin substrates, fibrins are insoluble fibrous non globular proteins found in blood clots and in thrombosis condition(Excessive generation of fibrin due to activation of the coagulation cascade leads to thrombosis). fibrinolytic proteases are hydrolyze both native and denatured Fibrin, and the enzymes are widely used not only in chemical and medical industries but also in food and basic biological science**[3]**.In this study an attempt was made for the screening and isolation of fibrinolytic proteases producing bacteria from various regions of Bangalore.

MATERIALS AND METHODS

Collection and isolation of sample

Samples were collected from dump yards of beef, chicken, fish and milk centers at Solddevanahalli, Chikkabanavara, Devasandra, K.R.puram, Tannary road and Yashwanthpur in and around Bangalore, Karnataka, India. The samples were labeled after collected. These were spread onto isolation media (Fibrin plate agar) and incubated at 37^{o}C for 24 hours after serial dilution of 10^{-1} to $10^{-6.}$.

Screening of fibrinolytic proteases production by plate assay

The isolates were screened for fibrinolytic protease activity in triplets. This was done by inoculating the organisms on the modified Fibrin plate agar**[4]**. containing 1.2%w/v agarose,0.4%w/v human fibrinogen and 20 U/ml human thrombin in a petridisc and incubated at 37^{o}C for 24 hours. A clear zone around the growth of the bacteria was indicated to fibrinolytic proteases activity

Table -1:Tabulation for Samples Description

S.NO	DESIGNATION OF SAMPLE	SAMPLE COLLECTED AREA	SAMPLE COLLECTED LAND MARK	SAMPLE NATURE	SAMPLE PH
1	ABMRCP -1	Shivaji Nagar	Opposite to Maszid at Chiken center	Semisolid sticky Seems to Brown in colour	7.64
2	ABMRCP -2	Tannery Road	Near to Bus stop at Chiken center	Semisolid Seems to Black in colour	7.60
3	ABMRCP -3	Tannery Road	Near to Bus stop Chiken Center	Semisolid Seems to Brown in colour	7.72
4	ABMRCP -4	Tannery Road	Slaughter house opposite canal	Hard consist of sand and clay seems to Brown in colour	7.65
5	ABMRCP -5	Solddevanahalli	Near to Bus stop Chiken Center	Semisolid Seems to Brown in colour	7.62
6	ABMRCP -6	Chikka Banavara	Near to Bus stop Chiken Center	Sticky consist of sand and clay seems to Brick red in colour	7.44
7	ABMRCP -7	K.R.Puram	Devasandra lake Chiken dump	Semisolid Seems to red in colour	7.71
8	ABMRCP -8	Tin Factory	Opposite to Maszid at Chiken center	Semisolid Seems to red in colour	7.60
9	ABMRCP -9	Tin Factory	Near to Bus stop Chiken Center	Hard consist clay seems to Black in colour	7.26
10	ABMRCP -10	Yashwanth Pura	Fish market Near to Railway station	Sticky consist of sand and clay seems to Black	7.34

Isolates on Fibrin plate agar Pure culture in petridish Pure culture in slants

Figure : fibrinolytic proteases production and pure cultures

Identification of Bacteria

The isolated bacteria were identified based on cellular morphology, growth condition, grams staining, endospore staining, capsule staining and biochemical tests **[5]**.

RESULTS AND DISCUSSION

Six bacterial isolates were obtained(Table:3) from soil samples of ABMRCP 1 to ABMRCP 10 (Table:1)and identified as *Bacillus cerus, Bacillus licheniformis* and *Staphylococcus aureus.* Morphologically and biochemically. The colonies were subjected to grams staining,capsule staining and endospore staining.The colonies which were positive and negative for Grams staining,Capsule and endospore

staining were considered for further studies(Table 3&4).The selected colonies were streaked on fibrin plate agar .The plates were subjected to incubation for a period of 24 hours at 37^{o}C.The plates which showed clear zone around the streaked area of test organism was selected as fibrinolytic proteases producing strain.The organisms named(Table2) showed the inhibition zone and was subjected to various biochemical tests(Table4). G isolates (Table2) showed the following results for the biochemical tests.These were positive for Methyl red test,Starch hydrolysis,Citrate utilization test.Oxidase test,gelatin hydrolysis test.urease test and nitrate reduction test ,and few isolates were shows negative for Voges Paskauer test,Indole test and Catalase test.After biochemical tests these organisms were confirmed to belong to the Bacillus and Cocci species (*Bacillus cerus , Bacillus licheniformis* and *Staphylococcus aureus*) which shows the capability of producing fibrinolytic protease.

Table -2:Tabulation for results of colony characteristics which shows fibrinolytic proteases activity.

STRAIN NO.	COLONY SURFACE	COLONY COLOUR	VISUAL CHARACTERISTICS	SHAPE OF THE COLONY	HEIGHT OF THE COLONY	FIBRINOLYTIC/GELATINASE ACTIVITY
G-1	Smooth	Brown	Opaque	Irregular	Raised	Positive
G-2	Smooth	Off white	Translucent	Circular	Raised	Positive
G-3	Smooth	Brown	Translucent	Irregular	Flat	Positive
G-4	Smooth	Off white	Opaque	Irregular	Raised	Positive
G-5	Smooth	Brown	Opaque	Irregular	Raised	Positive
G-6	Smooth	Brown	Translucent	Irregular	Flat	Positive

Table -3: Tabulation for results of Staining Techniques.

STRAIN NO.	GRAM STAINING	MORPHOLOGY (BACILUS/COCCI)	ENDOSPORE STAINING	CAPSULE STAINING
G-1	Positive	Rods	Positive	Positive
G-2	Positive	Rods	Positive	Positive
G-3	Positive	Cocci	Positive	Positive
G-4	Positive	Rods	Positive	Positive
G-5	Positive	Rods	Positive	Positive
G-6	Positive	Cocci	Positive	Positive

Table -4: Tabulation for results of Various Biochemical tests

S.No.	SAMPLES	INDOLE	MR	VP	AMYLASE	NITRATE	OXIDASE	CATALASE	UREASE	GELATINASE	FIBRINOLYTIC ACTIVITY
1	**G-1**	**+Ve**	**-Ve**	**-Ve**	**+Ve**	**-Ve**	**+Ve**	**+Ve**	**+Ve**	**+Ve**	**+Ve**
2	**G-2**	**-Ve**	**-Ve**	**+Ve**	**+Ve**	**+Ve**	**-Ve**	**-Ve**	**+Ve**	**+Ve**	**+Ve**
3	**G-3**	**-Ve**	**+Ve**	**+Ve**	**-Ve**	**+Ve**	**-Ve**	**+Ve**	**-Ve**	**+Ve**	**+Ve**
4	**G-4**	**-Ve**	**-Ve**	**+Ve**	**+Ve**	**+Ve**	**-Ve**	**-Ve**	**+Ve**	**+Ve**	**+Ve**
5	**G-5**	**+Ve**	**-Ve**	**-Ve**	**+Ve**	**-Ve**	**+Ve**	**+Ve**	**+Ve**	**+Ve**	**+Ve**
6	**G-6**	**-Ve**	**+Ve**	**+Ve**	**-Ve**	**+Ve**	**-Ve**	**+Ve**	**-Ve**	**+Ve**	**+Ve**

CONCLUSION

The search for promising strains of fibrinolytic proteases producers is a continuous process. The isolates which shows higher fibrinolytic proteases activity were selected for biochemical characterization and identification. The organisms were identified as *Bacillus cerus ,Bacillus licheniformis* and *Staphylococcus aureus*. on the basis of data obtained in the present work it can be concluded that *Bacillus* and *Cocci* species isolates can be employed in the production of fibrinolytic proteases.

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