IJPSR (2013), Vol. 4, Issue 9 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 08 May, 2013; received in revised form, 26 June, 2013; accepted, 26 August, 2013; published 01 September, 2013

SCREENING AND ISOLATION OF FIBRINOLYTIC PROTEASE PRODUCING MESOPHILIC BACTERIA FROM SLAUGHTER HOUSES IN BANGALORE

E. Venkata Naga Raju* and G. Divakar

Department of Biotechnology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur- 522 510, Andhra Pradesh, India
Department of Biotechnology & Microbiology, Acharya & B.M. Reddy College of Pharmacy, Soldevanahalli, Hesaraghatta, Bangalore- 560 090, Karnataka, India

Keywords:

Fibrinolytic Protease, Slaughter houses, Fibrin plate, *Bacillus cereus, Bacillus circulans, Pseudomonas aeruginosa*

Correspondence to Author:

E. Venkata Naga Raju

Department of Biotechnology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur- 522 510, Andhra Pradesh, India

E-mail: venkatanagarajue@gmail.com

ABSTRACT: This work has been undertaken for the screening and isolation of fibrinolytic protease producing mesophilic bacteria from ten soil samples, collected from slaughter houses 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 5 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 cereus, Bacillus circulans, Pseudomonas aeruginosa, Pseudomonas fluorescens* and *E.coli*.

INTRODUCTION: Enzymes are delicate protein molecules necessary for life. Proteolytic enzymes are ubiquitous in occurrence, being found in all living organisms, and are essential for cell growth and differentiation [1]. The extracellular proteases are commercial value and find multiple applications in various industrial sectors. Although there are many microbial sources available for producing proteases, only a few are recognized as commercial producers [2]. Of these, strains of *Bacillus sp*. dominate the industrial sector [3].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.4(9).3625-29

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.4(9).3625-29

Fibrinolytic enzyme is well known as a sub class of protease, which has an ability to degrade fibrin [4, 5, 6, 7, 8]. Blood clots (fibrin) are formed from fibrinogen by thrombin (EC 3.4.21.5) and are lysed by plasmin (EC 3.4.21.7), which is activated from plasminogen by tissue plasminogen activator [9]. Deposition of fibrin in blood vessels normally increases thrombosis, resulting in myocardial infarction and other cardiovascular diseases [10, 11].

Fibrinolytic proteases are the single class of enzymes which play an important part in the metabolism of many microorganisms like species of *Pseudomonas* [12], *staphylococcus* [13], *Alteromonas* [14], *Coryneform bacteria* [15], *Penicillium* [16], *Asperigillus* [17, 18, 19], *Fusarium* [20, 21], *Trichotecium* [22], *Actinomyces* [23, 24], *Streptomyces* [25, 26], *Escherichia coli* [27] and *Bacillus* [28, 29, 30, 31, 32, 33, 34].

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 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. The genus *Bacillus* contains a number of industrially important species and approximately half of the present commercial production of bulk enzymes derives from the strains of *Bacillus*.

In this study, an attempt was made for the screening and isolation of fibrinolytic protease producing mesophilic bacteria from slaughter houses various regions of Bangalore.

MATERIALS AND METHODS:

Collection and isolation of sample: Samples were collected from slaughter houses of beef, chicken and fish at Soldevanahalli, 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°C for 24 hours after serial dilution of 10^{-1} to 10^{-6}.

TABLE 1: TABULATION FOR SAMPLES DESCRIPTION

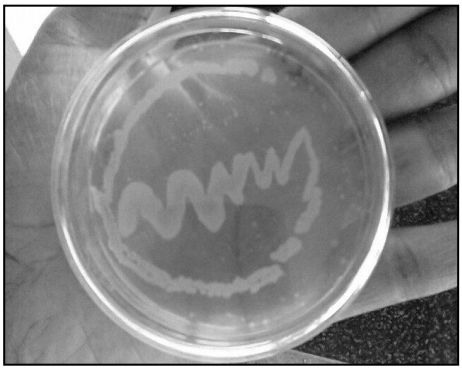
S. No.	Designation of sample	Sample collected area	Sample collected land mark	Sample nature	Sample pH
1	ABMRCP -1	Shivaji Nagar	Opposite to Maszid at Chiken center	Semisolid sticky Seems to Brown in color	7.68
2	ABMRCP -2	Tannery Road	Near to Bus stop at Chiken center	Semisolid Seems to Black in color	7.80
3	ABMRCP -3	Tannery Road	Near to Bus stop Chiken Center	Semisolid Seems to Brown in color	7.72
4	ABMRCP -4	Tannery Road	Slaughter house opposite canal	Hard consist of sand and clay seems to Brown in color	7.80
5	ABMRCP -5	Solddevanahalli	Near to Bus stop Chiken Center	Semisolid Seems to Brown in color	7.72
6	ABMRCP -6	Chikka Banavara	Near to Bus stop Chiken Center	Sticky consist of sand and clay seems to Brick red in color	7.44
7	ABMRCP -7	K.R.Puram	Devasandra lake Chiken dump	Semisolid Seems to red in color	7.71
8	ABMRCP -8	Tin Factory	Opposite to Maszid at Chiken center	Semisolid Seems to red in color	7.60
9	ABMRCP -9	Tin Factory	Near to Bus stop Chiken Center	Hard consist clay seems to Black in color	7.26
10	ABMRCP -10	Yashwanth Pura	Fish market Near to Railway station	Sticky consist of sand and clay seems to Black	7.34

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 [35]. Containing 1.2%w/v agarose, 0.4%w/v human fibrinogen and 20U/ml human thrombin in a petridisc and incubated at 37°C for 24 hours. A clear zone around the growth of the bacteria was indicated to fibrinolytic proteases activity.

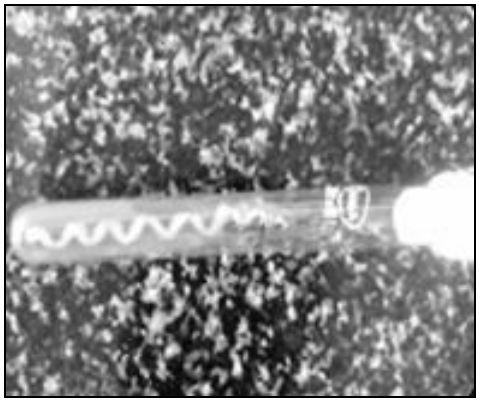
TABLE 2: TABULATION FOR RESULTS OF COLONY CHARACTERISTICS WHICH SHOW FIBRINOLYTIC PROTEASES ACTIVITY

Strain no.	Colony surface	Colony color	Visual characteristics	Shape of the colony	Height of the colony	Fibrinolytic activity
GD-15	Smooth	Brown	Opaque	Irregular	Raised	Positive
GD-25	Smooth	Off white	Translucent	Circular	Raised	Positive
GD-35	Smooth	Brown	Translucent	Irregular	Flat	Positive
GD-45	Smooth	Off white	Opaque	Irregular	Raised	Positive
GD-55	Rough	White	Opaque	Irregular	Flat	Positive

ISOLATES ON FIBRIN PLATE AGAR



PURE CULTURE IN PETRIDISH



PURE CULTURE IN SLANTS

FIGURE 1, 2, AND 3: 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 [36, 37].

TABLE 3: TABULATION FOR RESULTS OF STAINING TECHNIQUES

Strain no.	Gram staining	Morphology (Bacilus/Cocci)	Endospore staining	Capsule staining
GD-15	Negative	Rods	Negative	Positive
GD-25	Positive	Rods	Positive	Positive
GD-35	Negative	Rods	Negative	Positive
GD-45	Negative	Rods	Negative	Positive
GD-55	Positive	Rods	Positive	Positive

TABLE 4: TABULATION FOR RESULTS OF VARIOUS BIOCHEMICAL TESTS

Strain no.	Indole	MR	VP	Amylase	Nitrate	Oxidase	Catalase	Urease	Gelatinase	Fibrinolytic Activity
Pseudomonas aeruginosa GD-15	+Ve	-Ve	+Ve	+Ve	-Ve	+Ve	-Ve	+Ve	+Ve	+Ve
Bacillus circulans GD-25	-Ve	+Ve	-Ve	+Ve	-Ve	+Ve	+Ve	-Ve	+Ve	+Ve
Pseudomonas fluorescens GD-35	+Ve	-Ve	+Ve	+Ve	-Ve	+Ve	-Ve	-Ve	+Ve	+Ve
E. coli GD-45	+Ve	+Ve	-Ve	+Ve	+Ve	-Ve	+Ve	-Ve	+Ve	+Ve
Bacillus cereus GD-55	-Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	+Ve

RESULTS AND DISCUSSION: Five bacterial isolates were obtained (**Table 3**) from soil samples of ABMRCP 1 to ABMRCP 10 (Table 1), identified morphologically and biochemically as *Bacillus cereus, Bacillus circulans, Pseudomonas aeruginosa, Pseudomonas fluorescens* and *E. coli*. 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 were selected as fibrinolytic proteases producing strain.

The organisms named (Table 2) showed the inhibition zone and were subjected to various biochemical tests (Table 4).

GD isolates (Table 2) 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* species (*Bacillus cereus, Bacillus circulans, Pseudomonas aeruginosa, Pseudomonas fluorescens* and *E. coli*) which shows the capability of producing fibrinolytic protease.

CONCLUSION: Keeping in view the industrial importance of fibrinolytic proteases, researchers are more and more focusing on discovering fibrinolytic proteases with novel properties to meet the industrial requirements as well as increasing demand of global enzyme market. The search for promising strains of fibrinolytic proteases producers is a continuous process. The isolates which show higher fibrinolytic proteases activity were selected for biochemical characterization and identification. The organisms were identified as *Bacillus cereus, Bacillus circulans, Pseudomonas aeruginosa, Pseudomonas fluorescens* and *E.coli*. On the basis of data obtained in the present work it can be concluded that *Bacillus* species isolates can be employed in the production of fibrinolytic proteases.

ACKNOWLEDGMENTS: The authors would like to thank the chairman Acharya Institutes, Sri B. Premnath Reddy, for providing laboratory facilities and supporting this research work.

REFERENCES:

1. Sharma S, Aneja MK, Mayer J, Scholter M and Munch JC: RNA fingerprinting of microbial community in the rhizosphere soil of grain legumes. Fems Microbiol Lett 2004; 240: 181-186.
2. Gupta R, Beeg Q, Khan S and Chauhan B: An overview on fermentation, downstream processing and properties of microbial alkaline proteases. Appl Microbiol Biotechnol 2002; 60(4): 381-395.
3. Gupta R, Beeg Q and Loranz P: Bacterial alkaline protease molecular approaches and industrial applications. Appl Microbiol Biotechnol 2002; 59(1): 15-32.
4. Fujita M, Nomura K, Hong K, Ito Y, Asada A and Nishimuro S: Purification and character of a strong fibrinolytic enzyme (nattokinase) in the vegetable cheese natto, a popular soybean fermented food in japan. Biochem Biophys Res Commun 1993; 197: 1340-1347.
5. Jeong YK, Yang WS, Kim KH, Chung KT, Loo WH and Park JU: Purification of a fibrinolytic enzyme (myulchikinase) from pickled anchovy and its cytotoxicity to the tumer cell lines. Biotechnol Lett 2004; 26:393-397.
6. Leonardi A, Gubensek F and Krizaj I: Purification and characterization of two hemorrhagic metalloproteinases from the venom of the long nosed viper, *Vipera ammodytes ammodytes*. Toxicon 2002; 40:55-62.
7. Sumi H, Nakajima N and Yatagai C: A unique strong fibrinolytic enzyme (katsuwokinase) in skipjack Shiokara a Japanese traditional fermented food. Comp Biochem Physiol Biochem Mol Biol 1995; 112:543-547.
8. Wong AH and Mine Y: Novel fibrinolytic enzyme in fermented shrimp paste, a traditional asian fermented seasoning. J Agric Food Chem 2004; 52: 980-986.
9. Voet D and Voet JG: Biochemistry, Wiley, New York 1990: 87-95.
10. Bode C, Runge MS and Smalling RW: The future of thrombolysis in the treatment of acute myocardial infarction. Eur Heart J 1996; 17:55-60.
11. Yoshinori M, Ado HO, Wong K and Jiang BO: Fibrinolytic enzymes in Asian traditional fermented foods. Food Research International 2005; 38(3): 243-250.
12. Imshenetskii AA, Demina NS, Lysenko SV and Evdokimova MD: Fibrinolytic activity of bacteria from *Pseudomonas* genus. Prikl Biokhim Microbiol 1991; 27:845-849.
13. Berdzulishvili EM, Afanaseva TI and Alergant AP: Fibrinolytic activity of pathogrnic *staphylococci* of different origins. Lab Delo 1973; 6:332-334.
14. Demina NS, Veslopolova F and Gaenko GP: The marine bacterium *Alteromonas piscicida*-a producer of enzymes with thrombolytic action. Izv Akad Nauk Biol 1990; 3:415-419.
15. Egorov NS, Landau NS and Milovanova II: Fibrinolytic activity in mono and mixed cultures of *Coryneform* bacteria. Nauchnye Doki Vyss Shkoly Biol Nauki 1982; 10:86-90.
16. Andreeva NA, Ushakova VI and Egorov NS: Study of proteolytic enzymes of various strains of *penicillium lilacinum* in relation to their fibrinolytic activity. Mikrobiologiia 1972; 4:417-422.
17. Larcher G, Bouchara JP and Tronchin G: Purification and characterization of a fibrinogenolytic serine proteinase from *Asperigillus fumigates* culture filtrate. Febs Lett 1992; 10:65-69.
18. Ushakova VI, Dalko LD and Egorov NS: Study of the protease complex synthetized by *Aspergillus kanagawaensis* in relation to its fibrinolytic activity. Nauchnye Doki Vyss Shkoly Biol Nauki 1974; 8:93-97.
19. Klocking HP and Markwardt F: Thrombolytic and pharmacodynamic properties of *Asperigillus ochraceus* protease. Farmakol Toksikol 1975; 38:341-349.
20. Abdel Fattah AF, Ismail AS and Saleh SA: Purification and properties of two fibrinolytic enzymes from *Fusarium oxysporum* N.R.C.I. Zentralbl Mikrobiol 1993; 148:123-128.
21. EI-Aassar SA: Production and properties of fibrinolytic enzyme in solid state cultures of *Fusarium pallidoorosem*. Biotechnol Lett 1995; 17:943-948.
22. Stepanova TSN, Maksimova RA, Lulikova EP and Silaev AB: Fractionation of a preparation of fibrinolytic enzymes tricholysin formed from *Trichotecium roseum* LK. Ex Fr.on carboxymethyl-sephadex C-50. Prikl Biokhim Mikrobiol 1976; 12:407-410.

23. Egorov NS, Kochetov GA and Khaidarova NV: Isolation and properties of the fibrinolytic enzyme from *Actinomyces thermovulgaris* culture broth. Mikrobiologiia 1976; 45:455-459.
24. Egorov NS, Alnuri MA and Krivova AI: Optimization of the nutrient medium for *Actinomyces spheroids* producer of proteolytic enzymes with fibrinolytic activity. Mikrobiollogiia 1976; 45: 607-613.
25. Egorov NS, Kochetov GA and Khaidarova NV: Physicochemical properties of a fibrinolytic enzyme from *Streptomyces thermovulgaris* culture broth. Nauchnye Doki Vyss Shkoly Biol Nauki 1986; 4: 89-94.
26. Chitte RR and Dey S: Potent fibrinolytic enzyme from a thermophilic *Streptomyces megaspores* strain SD5. Lett Appl Microbiol 2000; 31:405-410.
27. Malke H and Ferretti: Streptokinase cloning expression and excretion by *Escherichia coli*. Proc Natl Acad Sci USA 1984; 81:57-61.
28. Yu R, Qi H, Zhang T and Wu WT: Preliminary studies on in vitro and in vivo thrombolytic activities of thrombolytic enzyme from an induced *Bacillus subtilis* strain. Schuan Da Xue Xue Bao Yi Xue Ban 2005; 36:93-96.
29. Cherkessova GV, Nesterova NG and Fetisova ZS: The thrombolytic activity of *Bacillus mesentricus* at different conditions of nitrogen nutrition. Mikrobiologiia 1989; 58:915-919.
30. Fayek KI and Eisayed ST: Some properties of two purified fibrinolytic enzymes from *Bacillus subtilis* and *Bacillus polymyxa*. Zallg Microbiol 1980; 20:383-387.
31. Vyrbornykh CN, Landau NS and Egorov NS: Biosynthesis of fibrinolytic enzymes with different mechanisms of action by microorganisms of genus *Bacillus.* Microbiologiia 1990; 59:782-789.
32. Egorov NS, Iudina TG, Loriia ZHK and Kreier VG: Fibrinolytic activity of several variants of *Bacillus thuringiensis*. Prikl Biokhim Microbiol 1979; 15: 416-420.
33. Peng Y, Huang Q, Zhang, RH and Zhang YZ: Purification and characterization of a fibrinolytic enzyme produced by *Bacillus amyloliquefaciens* DC-4 screen from douche, a traditional Chinese soybean food. Comp Biochem Physiol Biochem Mol Biol 2003; 134: 45-52.
34. Sumi H, Hamada H, Tsushima H and Mihara H: A Novel fibrinolytic enzyme (Nattokinase) in the vegetable cheese Natto a typical and popular Soybean food in the Japanese Diet. Experientia 1987; 43(10): 1110-1111.
35. Astrup T and Mullertz S: The fibrin plate method for estimating fibrinolytic activity. Arch Biochem Biophys 1952; 40: 346–351.
36. Sneath HAP and Halt GJ: Bergey's manual of systematic bacteriology Vol. 2 Baltimore, MD, Williams and Wolkins 1986.
37. Pokorny M, Vitale V and Zuvanic J: Bergys manual of Determinative Bacteriology *Streptomyces rimoses* extacellular protease Characterization and evaluation of various crude preparations, Europe. J Appl Microbiol Biotechnol 1979; 8: 81-90.

How to cite this article:
Raju EVN and Divakar G: Screening and isolation of Fibrinolytic protease producing Mesophilic bacteria from slaughter houses in Bangalore. *Int J Pharm Sci Res* 2013: 4(9); 3625-3629. doi: 10.13040/IJPSR. 0975-8232.4(9).3625-29