Original Article

Clot Buster Enzyme Activity from *Bacillus* sp.,

Mohanasrinivasan Vaithiyalingam*, Subin Jacob Chacko, Nivetha Anbalagan, Jannatul Firdous Siddique

School of Bio Sciences and Technology, VIT University, Vellore, Tamil Nadu, INDIA.

ABSTRACT

Context: Clot buster enzyme is widely used as the thrombolytic agent due to its low cost as compared to several other thrombolytic agents. **Aim:** The aim of the study is to isolate clot buster enzyme producing bacteria from soil sample. **Materials and Methods:** In the present study, we have isolated 20 different bacterial strains from soil source collected near meat shops and screened for the production of clot buster enzymes. The sample was serially diluted and characterized in the *Bacillus* differential medium. Most promising clot buster enzymes producing bacterial strain were selected by the initial screening of the casein hydrolase assay. The one strain showed activity with a zone of clearance in the casein hydrolase assay were characterized by various morphological and biochemical test. Morphological identification of the bacterial cells was examined by Gram staining and by SEM analysis. Production, media was prepared and inoculated by potent strain VITSM04 and processed for seven days of fermentation. The enzyme was purified by 70% ammonium sulphate precipitation, 50kDa ultra filtration and gel filtration by Sephadex G -100. **Results:** The crude enzyme was found to show strong clot buster enzyme activity with specific activity of 640.80U/mg in clot lysis of human blood. The concentration of the protein was found to be 55.12mg/ml. The molecular weight of clot buster enzyme was detected by 10% SDS-PAGE in single band with low molecular weight of 28kDa. Clot buster enzyme was quantified using an HPLC system with retention time of 4 min by the refractive index detector. **Conclusion:** Thus the enzyme can be used in the release of blood clot and fibrinolysis disorder which is a serious medical problem. The successful development of potent strain VITMS07 can holds the promising clot buster enzyme activity can be used in industrial application with large scale production.

Key words: Cardiovascular, Clot buster, Casein hydrolysis, SDS-PAGE, HPLC.

INTRODUCTION

Globally today Cardiovascular Related Diseases (CVDs) is one of the major threaten issue where the quality of life decrease in humans.[1] Cardiovascular diseases is an abnormal function of the heart or blood vessels that includes heart attacks,[2] myocardial infarction,[3] pulmonary embolism[4] stroke and cardiac rhythm problems[1] etc, Enzymes are biocatalysts produced in human body from amino acids, which initiates and regulates countless biologic, metabolic and digestive reactions. Enzymes are catalytic and convert multiple target molecules to the desired products.[3] Fibrinolysis enzyme created more attention for cardiovascular disease and clot lysis. In 1960, many clot buster enzymes such as urokinase, streptokinase and tissue Plasminogen Activator (t-PA) were used for clinical treatment of CVDs.[5] The role of clot buster enzyme is to cleave the clot, blocking the blood vessels and relive the CVDs. This property makes clot buster enzyme more specific and potent drugs that can achieve their utility as a therapeutic agent against the CVDs.[6]

Nattokinase is one of the clot buster enzyme has more advantages over other commercially used medicine in the treatment of CVDs. This enzyme can be easily administered orally which gives rise to mild and frequent enhancement of the

Submission Date: 18-07-2019;
Revision Date: 05-11-2019;
Accepted Date: 26-02-2020

DOI: 10.5530/ijper.54.2s.96

Correspondence:
Dr. V Mohanasrinivasan
Associate Professor
Department of Biomedical Sciences School of Bio Sciences and Technology
Vellore Institute of Technology (VIT), Vellore-632014, Tamil Nadu, INDIA.
Phone: +91 9486802902
E-mail: v.mohan@vit.ac.in

www.ijper.org

fibrinolytic activity in the plasma showing stability in the gastrointestinal tract.[1,5] Furthermore, some clot buster activity was retained in the blood for more than 3 h.[7-9] Clot buster enzymes have been extracted and identified from snakes, earthworms and bacteria: *Streptococcus pyogenes, Aeromonas hydrophila, Serratia* E15, *B. natto*, *Bacillus amyloliquefacens*, *Staphylococcus aureus,* Actinomycetes and fungi: *Fusariums* sp; *Mucor* sp, *Armillaria mellea*.[10] Strpetokinase,[1] Nattokinase, protease,[11] Actinokinase (marine), Urokinase[5] are some of the clot buster enzymes were previously reported in research papers. Bromelain is also proteolytic enzyme that is typically extracted from fruit, stem and leaves of Pineapple (*Ananuscomosus)* plant. Pineapple being an epiphyte utilizes these proteolytic enzymes (Bromelain) to break down organic compounds and obtain its supply of nitrogen and phosphorus.[12] Hence the current study focused on purification of clot buster enzyme from *Bacillus* sp., isolated from soil sample and to determine its clot lysis activity against human blood.

Sample collection and Isolation of *Bacillus*

Collection of samples

The soil sample was collected from the meat shop in Vellore, Tamil Nadu.

The soil was chosen from the upper layer of the soil at 0.5° by 0.5° latitude/longitud, as most of the bacteria were concentrated there. Approximately, 1g of sample were dissolved in 100mL of sterile distilled water and kept in a shaker to incubate for 30 mins at 120rpm. The suspension was serially diluted from 10^{-1} to 10^{-5} and by spread plate technique it was spreaded on *Bacillus* differential media and incubated at 37°C for 24hr.[13]

Further by casein hydrolytic assay, the colony of different morphology was selected and screened for clot buster enzyme. Sub culturing was done on dilute nutrient agar to isolate pure cultures.

Casein hydrolysis assay for the isolates

Isolates were screened for their ability for protease production on nutrient agar medium supplemented with 2% of casein and human serum and followed with incubation for 24hr at 37°C.[14]

Production media

The production media was prepared by g/100 mL: Glucose - 0.5 g, Yeast Extract - 0.5 g, KH_2PO_4 - 0.25 g, $MgSO_4 \cdot 7H2O$ 0.04 g, $NaHCO_3$ - 0.1 g, $CH_3COONa \cdot 3H2O$ - 0.1 g, $FeSO_4 \cdot 7H2O$ - 0.002 g, $MnCl_2 \cdot 4H2O$ - 0.002 g. The pH was adjusted to 7.0 ± 0.5. Bacterial suspension was inoculated and incubated at 37°C in shaker incubator at 150 rpm for 24 hr.[3]

After 7 days, the cells were harvested via centrifugation for 20 min, 10,000 rpm at 4°C. The resultant cell mass is utilized further for extracellular enzyme extraction for clot buster enzyme activity.[1]

Partial purification of clot buster enzyme

Ammonium sulphate precipitation and Ultra-filtration

The *Bacillus* culture was transferred to production media and kept for incubation for 7 days, centrifuged at 10,000 rpm for 20 min and Ammonium sulphate at 0-70% saturation was added to the supernatant to precipitate the proteins.[10,15] The supernatant was centrifuged for 15 min at 5000 rpm at 4°C to recover protein. Partial precipitated protein obtained from ammonium sulphate precipitation dissolved in 50mM Tris buffer (pH 7.2) and carried out for ultra-filtration membrane (Amicon Ultra-15, Millipore, India) with a 50kDa molecular cut-off membrane. The ultra-filtration tube was centrifuged at 4000rpm for 20 min at 4°C. After centrifugation, the retentate and permeate was collected separately in tubes and were used for protein estimation and enzyme activity. Permeate was used for gel filtration chromatography.

Gel permeation chromatography and SDS-PAGE

In self-packed Sephadex G-100 column (1.6 in diameter, 25 cm gel bed height, 3 mL sample volume) equilibrated with sterile column buffer, 0.1 M Tris-HCL buffer (pH 7), the gel filtration was carried out.

The active fraction, permeate collected from ultrafiltration was loaded in the column G-100. The samples were eluted with the same buffer at a flow rate of 1.0 mL/min. The fraction eluted was collected for the determination of protein content and clot lysis activity.

The molecular weight of the purified enzyme was determined by 10% SDS–PAGE and and compared the results with standard protein marker (20-206 kDa) obtained from SRL.

Determination of protein estimation and clot buster enzyme activity

To estimate the protein concentration of the crude and partially purified enzyme extracts, the Lowry's method[16] was used and clot buster enzyme activity was checked by clot lysis assay

In-vitro blood clot lysis assay

From the volunteer the blood sample was collected to pre weigh sterile eppendorf tube (200µL/tube) and incubated for clot formation at 37°C for 45 min.

After that gently the serum was separated from the clot and weight of clot in respective tube was determined.

The crude enzyme extract (100µl), ammonium sulfate precipitated, ultra-centrifuged and eluted protein from gel permeation chromatography from potent strain were added to the clots and Incubated for 90 min at 37°C and clot lysis was further observed. After clot disruption, the fluid were removed and the remaining clot was weighed to compare the difference with the control. Difference in weight between the clot lysed and untreated clot was noted and expressed as percentage of clot lysis according to the formula given below.[17,18]

$$\text{Clot weight} = \text{Weight of clot containing tube} - \text{Weight of tube alone}$$

$$\text{Percentage (\%) of clot lysis} = \frac{\text{Weight of the lysis clot}}{\text{Weight of clot before lysis}} \text{x100}$$

The one enzyme unit is expressed as, the enzyme which completely liquefies 1mL of clotted blood.[18]

Characterization of potent isolate VITSM04

The potent isolate which showed high clot lysis activity were characterized for biochemical analysis such as Gram stain, catalase test, indole test, MR-VP test, triple sugar ion test and nitrate reduction test. Biochemical characterization results were compared with Bergey's manual to.[19]

Scanning Electron Microscope (SEM)

Aliquots (5 ml) of freshly grown bacterial cultures were incubated for 16 ± 2 hr at 37°C were harvested, fixed, dehydrated and essentially embedded respectively according to McDourgall *et al.* 1994.[20] Microscopic analysis was carried out with scanning electron microscope (Leo Electron Microscopy Ltd. Cambridge, UK) operated at 15 keV.

Hemolytic activity

The isolate was streaked on selectively 5% blood agar medium (Hi-Media, India) and allowed to incubate for 24 hr at 37°C.

High performance liquid chromatography (HPLC)

The purified active fractions were lyophilized and checked for homogeneity, after getting eluted from gel filtration chromatography. The reverse phase HPLC of analytical column (25cm x 4.6mm) was used for the same.

Using linear gradient, i.e 100% constituted of 0.1% TFA (solvent A) and 70% acetonitrile to 20% dist. water (Solvent B), for 40 min was run. It was monitored at 220nm absorbance.

RESULTS AND DISCUSSION

Isolation of *Bacillus* sp., from soil sample

Based on colony morphology 20 different strains were isolated from soil samples. The colony morphology was dry, flat and irregular, with lobate margins appearance were observed which had a round symmetry. Bacterial strains characterized for Gram's staining and catalase test. Out of 20 strains, only five strains (VITSM07, VITSM05, VITSM03, VITSM02 and VITSM06) were shown to be gram positive bacilli with a long and short rod-shaped bacteria, forming small clumps, chains or single cells and fifteen were shown to be gram positive cocci. The isolate which showed gram positive rods for were carried out for the catalase reactions. All the 5 isolates were shown to be negative for catalase reaction. From the results, it was evident that bacilli were grown less number when compared to cocci. Preliminary characterization gram's reaction and catalase test shown in Table 1. Gram positive bacilli were screened to for extracellular enzyme through hydrolysis of casein (Figure 1).

Casein hydrolysis

Gram positive bacilli (VITSM2, VITSM3, VITSM5, VITSM6 and VTISM7) were screened for casein hydrolysis assay. One strain VITSM04 showed zone of

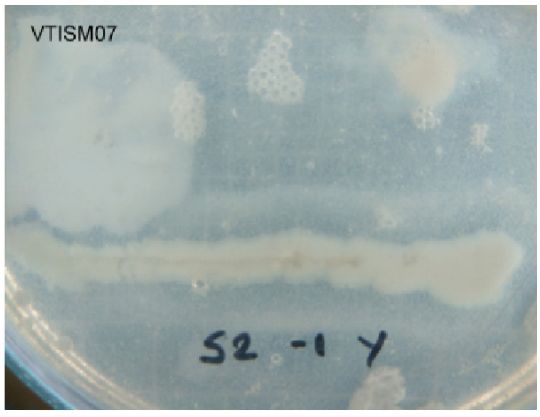


Figure 1: Zone of hydrolysis for casein hydrolysis test.

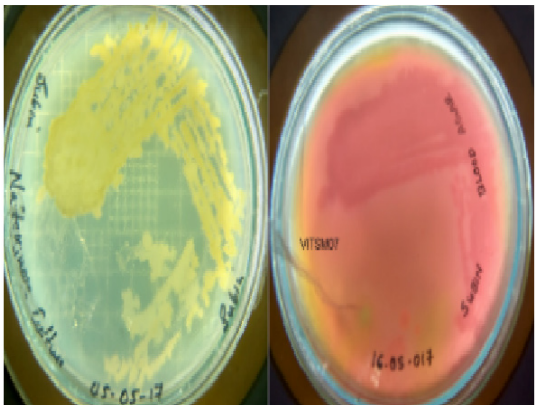


Figure 2: Potent strain *Bacillus* spp., on nutrient agar and blood agar plate.

Table 1: Morphological characterization of the bacterial isolates

Strain	Gram Staining	Shapes	Catalase test
VITSM01	Gram Positive	Cocci in chains	Positive
VITSM02	Gram Positive	Bacilli in short rods	Negative
VITSM03	Gram Positive	Bacilli in chains	Negative
VITSM04	Gram Positive	Cocci in chains	Positive
VITSM05	Gram Positive	Bacilli in short rods	Negative
VITSM06	Gram Positive	Bacilli in chains	Negative
VITSM07	Gram Positive	Bacilli in long rods	Negative
VITSM08	Gram Positive	Cocci in tetrads	Positive
VITSM09	Gram Positive	Cocci in chains	Positive
VITSM10	Gram Positive	Cocci in chains	Positive
VITSM11	Gram Positive	Cocci in tetrads	Positive
VITSM12	Gram Positive	Cocci in tetrads	Positive
VITSM13	Gram Positive	Cocci in tetrads	Positive
VITMS14	Gram Positive	Cocci in tetrads	Positive
VITSM15	Gram Positive	Cocci in chains	Positive
VITSM16	Gram Positive	Cocci in chains	Positive
VITSM17	Gram Positive	Cocci in chains	Positive
VITSM18	Gram Positive	Cocci in chains	Positive
VITSM19	Gram Positive	Cocci in chains	Positive
VITSM20	Gram Positive	Cocci in chains	Positive

hydrolysis for the casein hydrolysis assay (Figure 1). The isolated strain, showed yellow colored pigmentation on nutrient agar plate and ɣ hemolysis pattern on blood agar plate (Figure 2). The cultural, morphological and biochemical characteristics features confirms the strain VITSM04 belongs to the genus *Bacillus*. Colony morphology, microscopic and biochemical characterization was shown in Table 2. The results were similar to Subathradevi *et al*. 2015.[2]

SEM analysis of *Bacillus* sp., VITSM07

The scanning electron microscope was used to observe and identify the shape of the bacteria. The SEM analysis showed that the cells were rod shape at 18.00 KX and 9.00 KX magnifications. The length of the cells was 2 µm and the diameter was 10.5 mm (Figure 3).

Purification of clot buster enzyme from production media.

Ammonium sulphate precipitation saturated the protein at maximum of 70% according to standard ammonium sulphate table. According to Sunil L Harer, 2018 thermostable serine protease was precipitated up to 60% saturation.[21] Precipitated proteins were further continued with 50kDa ultra-filtration and permeate carried out for gel permeation chromatography. A total of 20 different (1mL/min) fractions was eluted from gel permeation chromatography and observed at 280nm to evaluate the protein presence. The protein fraction revealed maximum absorbance with 1.187 at 280nm

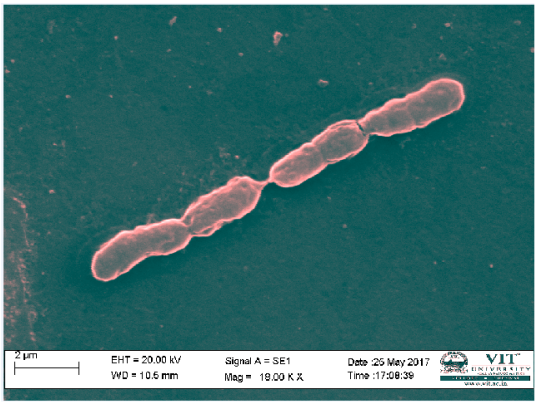


Figure 3: SEM analysis of *Bacillus* cells at 18.00 KX and 9.00 KX magnifications.

Table 2: Colony, Microscopic and Biochemical characterization of *Bacillus* spp., VITSM07.

Characterization	Tests	Results
Colony characterization	Colony Morphology, Blood agar medium	Small round colonies and ɣ hemolysis on blood agar plate
Microscopic characterization	Gram staining, Shape, Arrangement, Motility	Gram postive with long rods, chain formation and non motile
Biochemical characterization	Catalase test	Negative
	Indole test	Negative
	MR-VP test	Negative
	Triple sugar ion test	Positive
	Nitrate reduction test	Negative

which further continued with 10% SDS-PAGE. As a result the specific activity of the purified clot buster enzyme was found to be 1339.28U/mg. The purification fold of 2.0 with a yield of 15.92% was obtained. The similar reports were made by Mohanasrinivasan *et al.* 2014 for Staphylokinase from *Staphylococcus aureus* VITSDVM7 with specific activity of 1152U/mg with 12.8 yield percentages.[10] Summary of purification of the clot buster enzyme was shown in Table 3.

The 100 µl of enzyme showed complete liquefication within 90 min at room temperature.

The molecular weight was found to be 28kDa via SDS-PAGE (Figure 4). The results were similar to Subathra Devi Chandrasekaran *et al.* 2015.[2] They reported nattokinase were observed to be 21kDa from *Pseudomonas aeruginosa* in SDS-PAGE. JU HoKo *et al.* 2004 reported that the HPLC analysis of the clot buster enzyme extracted from *Bacillus subtillis* was found to be RT 9.06 min.[22] Similarly in the present research also we got a distinct peak at 4.118 and 9.429 min retention time which confirms the presence of clot buster enzyme (Figure 5)

Clot lysis activity

The crude enzyme, 70% precipitate from ammonium sulfate precipitation assay, permeate collected from 50kDa amicon ultrafiltration unit, protein fraction eluted from gel filtration at a volume of 100 µl showed clot lysis activity, as given in Figure 6.

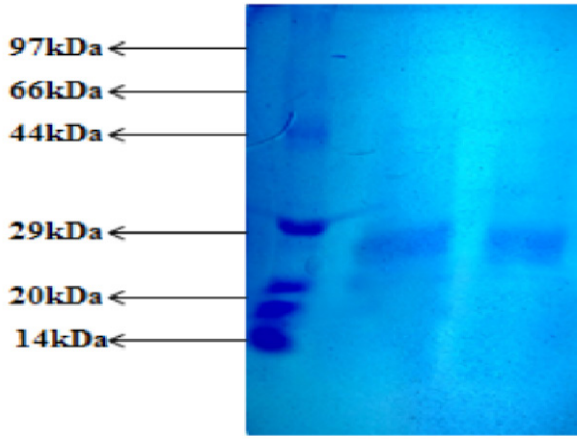


Figure 4: SDS-PAGE of purified clot buster enzyme from *Bacillus* sp.

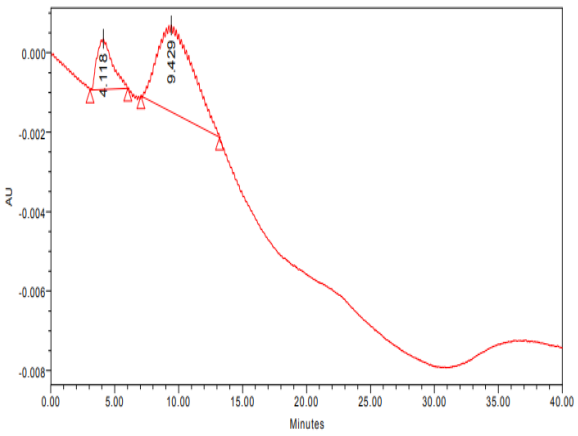


Figure 5: HPLC analysis for the clot buster enzyme.

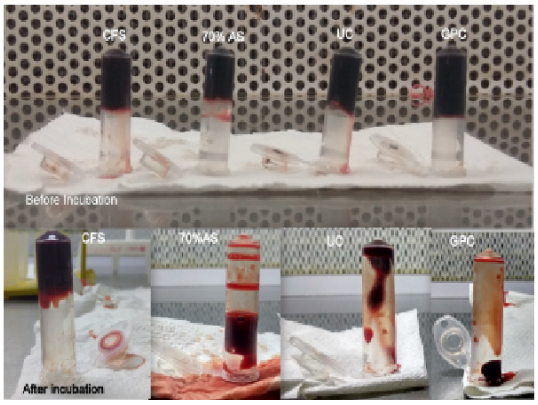


Figure 6: Clot buster activity.

CONCLUSION

From the results obtained in the study, it was concluded that *Bacillu* sp., grown in production media efficiently to produce clot buster enzyme which could be isolated from soil sample. Thus the enzyme can be used in the release of blood clot and fibrinolysis disorder which is a serious medical problem. The optimized fermented production medium showed enhanced production of

Table 3: Summary of purification of the clot-buster enzyme from *Bacillus* sp., (VITSM07).

Purification fraction	Total Volume	Total protein (mg)	Total enzyme activity (U/mg)	Specific activity (U/mg)	Purification fold	Yield (%)
CFS	100	55.12	35321	640.80	1	100
ASP	50	13.25	14235	1074.09	1.6	44.04
UF	10	6.1	7232	1185.57	1.8	20.47
GPC	3	4.2	5625	1339.28	2.0	15.92

CFS – Cell free supernatant; AS – Ammonium sulphate precipitation; UC – Ultracentrifugation; GPC – Gel permeation chromatography

purified clot buster specific enzyme activity 1339.28U/mg which was higher than the crude enzyme activity. In addition, 2.0 fold increase in enzyme production with 15.92% yield. The successful development of potent strain which is VITMS07 can holds the promising clot buster enzyme activity can be used in industrial application with large scale production.

ACKNOWLEDGEMENT

The authors thank VIT University for providing 'VIT SEED GRANT' for carrying out this research work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

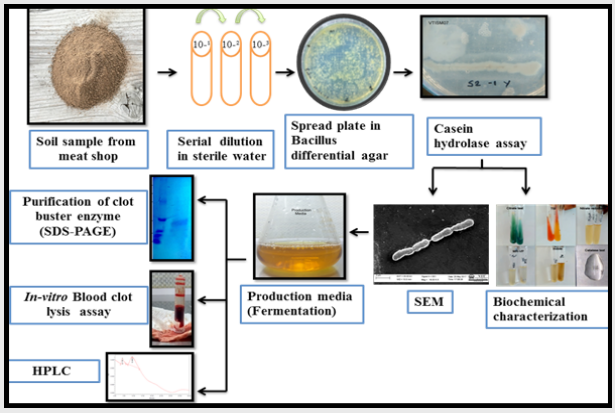
ABBREVIATIONS

CVD: Cardio Vascular Disease; **SEM:** Scanning Electron Microscope; **KH_2PO_4:** Monopotassium phosphate; **$MgSO_4$:** Magnesium Sulfate; **$NaHCO_3$:** Sodium bicarbonate; **CH_3COONa:** Sodium acetate; **$FeSO_4$:** Ferrous sulphate; **$MnCl_2$:** Manganese chloride; **SDS-PAGE:** Sodium dodecyl sulphate –Polyacrylamide gel electrophoresis; **HPLC:** High Performance liquid chromatography.

REFERENCES

1. Prashant C, Varad B, Supriya S. Media optimization for nattokinase production from a marine isolate at a shake flask level. International Journal of Advance Research in Science and Engineering. 2017;6:9.
2. Subathra DC, Mohanasrinivasan V, Ravi S, Sanjeev K, Swathi T, Vaishnavi B, *et al*. Exploring the *in vitro* Thrombolytic Activity of Nattokinase From a New Strain *Pseudomonas aeruginosa* CMSS. Jundishapur J Microbiol. 2015;8(10):e23567.
3. Vaishnavi B, Subathra DC. Exploring the *in vitro* thrombolytic potential of streptokinase-producing β hemolytic*Streptococci* isolated from bovine milk. J Gen Appl Microbiol. 2015;61(4):139-46.
4. Blix S. The effectiveness of activators in clot lysis, with special reference to fibrinolytic therapy: A new method for determination of preformed clot lysis. Acta Med Scand. 1962;172(S386):1-24.
5. Prafulla MM, Sagar VG, Smita SL. Production of Nattokinase using *Bacillus* natto NRRL 3666: Media Optimization, Scale Up and Kinetic Modeling. Food Sci Biotechnol. 2010;19(6):1593-1603.
6. Rand S, Omar S, El-Dahiyat F, Maha H, Sana S. Research advances in kinase enzymes and inhibitors for cardiovascular disease treatment. Future Sci OA. 2017;3(4):FSO204.
7. Sumi H, Hamada H, Nakanishi K. Enhancement of the fibrinolytic activity in plasma by oral administration of nattokinase. Acta Haemotol. 1990;84(3):139-43.
8. Suzuki Y, Kondo K, Matsumoto Y, Zhao BQ, Otsuguro K, Maeda T, *et al*. Dietary supplementation of fermented soybean, natto, suppresses intimal thickening and modulates the lysis of mural thrombi after endothelial injury in rat femoral artery. Life Sci. 2003;73(10):1289-98.
9. Fujita M, Hong K, Ito Y. Transport of nattokinase across the rat intestinal tract. Biol Pharm Bull. 1995;18(9):1194-6.
10. Mohanasrinivasan V, Subathra DC, Monidipa B, Jannatul FS, Pragya L, Jemimah NS, *et al*. Production of fibrinolytic Staphylokinase from UV Mutated *Staphylococcus aureus* VITSDVM7. International Journal of Chem Tech Research. 2014;6(8):4007-14.
11. El-Sabbagh SMM, Mohamed TS, Areeg IF. Characterization and Identification of some Actinomycetes producing protease enzyme in the aquatic habitat. Egypt J Exp Biol. 2012;8(2):161-73.
12. Moni P, Jacob K, Prithvi S, Arif AM, Sangeetha S. *In-vitro* Hemolytic and Clot Buster Activity of the Extracts of *Ananas comosus* (Pineapple). Int J Pharm Sci Rev Res. 2016;41(2):239-43.
13. Sreenivasulu P, Suman JDSD, Narendra K, Venkata RG, Krishna SA. Isolation and Identification of Probiotic Bacteria from River Banks of Krishna by Biochemical and Molecular Level Characterization. Int J Curr Microbiol App Sci. 2015;4(11):372-9.
14. Dubey R, Kumar J, Agrawala D, Char T, Pusp P. Isolation, production, purification, assay and characterization of fibrinolytic enzymes (Nattokinase, Streptokinase and Urokinase) from bacterial sources. African Journal of Biotechnology. 2011;10(8):1408-20.
15. Mohanasrinivasan V, Subathra DC, Ritusree B, Falguni P, Mohor M, Selvarajan E, *et al*. Enhanced production of nattokinase from UV mutated *Bacillus* sp. Bangladesh J Pharmacol. 2013;8(2):110-5.
16. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with Folin-Phenol reagent. J Biol Chem. 1951;193(1):265-75.
17. Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, Daginawala HF. Development of an *in vitro* model to study clot lysis activity of thrombolytic drugs. Thromb J. 2006;4:14.
18. Holmstrom B. Streptokinase assay on large agar diffusion plates. Acta Chem Scand. 1965;19(7):1549-54.
19. Holt NRK, Sneath HA, Stanley JT, Williams ST. Berge's Manual of Determinative Bacteriology. (9th ed), Baltimore; Wiliams and Wilkins, USA. 1994.
20. McDougall LA, Holzapfel WH, Schillinger U, Feely DE, Rupnow JH. Scanning electron microscopy of target cells and molecular weight determination of a bacteriocin produced by *Lactococcus lactis* D_{53}. International Journal of Food Microbiology. 1994;24(1-2):295-308.
21. Sunil LH, Manish SB, Neela MB. Isolation, purification and partial characterization of thermostable serine alkaline protease from a newly isolated *Bacillus thuringinsis*-SH-II-1A. African Journal of Biotechnology. 2015;17(7):178-88.
22. Ju HK, Jun PY, Yi PQ. Identification of two novel fibrinolytic enzymes from *Bacillus subtilis* QK02. Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology. 2004;137(1):65-74.

PICTORIAL ABSTRACT



About Authors

Dr.Vaithiyalingam Mohanasrinivan, Associate professor in the Department of Biomedical Sciences, School of BioSciences and Technology. His research area is the production of beta-galactosidase for probiotic drink formulation with low cost submerged fermentation, bacteriocin for anti-cancerous role and pediocin for antimicrobial activity from Lactic Acid Bacteria (LAB). Further, he have worked on the project, stain and clot buster enzyme such as streptokinase, urokinase, staphylokinase, nattokinase, etc producing bacteria, for industrial application and technical solution for improving animal health using biotechnological tools, Currently, He is focusing on the area molecular approach for the overproduction of Ramphacin from Actinomycetes hygroscopicus. In addition to his research work, he is guiding 3 internal and 2 external Ph.D. scholars to pursue their projects.

SUMMARY

The phenomenon like thrombosis occurs due to the accumulation of fibrin clot in blood vessels, which leads to cardiovascular diseases. The current study demonstrated the enhanced production of clot buster enzyme by *Bacillus* sp., isolated from soil sample.

- Among different isolates, one promising isolate VITMS07 *Bacillus* sp., was found positive for fibrinolytic protease screening on casein hydrolytic assay.
- The potent strain was further subjected for biochemical characterization, morphological size determination by SEM and haemolytic activity in blood agar plate.
- The enzyme was extracted and analysed for *in-vitro* blood clot lysis assay by holmstrom method.
- Purification of enzyme were carried out using ammonium sulfate, 50kDa ultrafiltration tube and finally by gel filtration chromatography using sephadex G-100.
- The molecular weight was determined via 10% SDS PAGE and found to be of 28kDa.
- HPLC analysis confirms the presence of clot buster enzyme with the peak at retention time of 9.429 compared with the earlier report.

Cite this article: Mohanasrinivasan V, Chacko SJ, Anbalagan N, Siddique JF. Clot Buster Enzyme Activity from *Bacillus* sp.,. Indian J of Pharmaceutical Education and Research. 2020;54(2s):s374-s380.