Partial Purification of Protease from *Bacillus licheniformis* and its Application as Thrombolytic Agent

Muhammad Asad Uz Zaman[1], Md. Arafat Al Mamun[2], Md. Abdul Mazid[1], Md. Mozammel Hoq[3]
[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, [2]Center for Advanced Research in Sciences, [3]Department of Microbiology, Dhaka university, Dhaka – 1000.

ABSTRACT

Thrombosis leads to myocardial infarction, stroke and other cardiovascular complications. Thrombolytic agents such as t-PA, u-PA, streptokinase etc are used to treat complications related to thrombosis. However, investigations are being pursued to find out new microbial enzymes as thrombolytics having better efficacy and specificity with less side effects, availability and affordability. To search for new thrombolytic proteases from microbial sources, mutant strain of *Bacillus licheniformis MZK05M9* was cultured in modified Urea-glucose medium at 37°C under shake culture conditions yielding 840.112 units/mg. The enzyme were partially purified using ammonium sulfate precipitation following ultrafiltration yielding 37713.922 units/mg. The molecular weight of the partially purified enzyme was 27.2 kDa and purification increased its specific activity to 16.5 fold with a recovery of 10%. The partially purified protease enzyme exhibited 32.84% thrombolytic activity, by *in vitro* clot lysis assay. The present results will be an useful basis for development of viable thrombolytic drugs to prevent or cure thrombosis and related disorders.

CONTACT

Muhammad Asad Uz Zaman
Department of Pharmaceutical Chemistry,
University of Dhaka, Dhaka – 1000.
Email : asad.accedu@gmail.com
Phone : +8801516105217
Web : https://bd.linkedin.com/in/zamanma

INTRODUCTION

Various types of thrombosis leads to cardiovascular diseases such as myocardial infarction, stroke, venous thromboembolism and other cardiovascular complications.

The available thrombolytic agents are two types, one is plasminogen activators, such as t-PA, urokinase, streptokinase. The other type is plasmin-like proteins. Streptokinase is a nonhuman protein, and its introduction into the circulatory systems can illicit severe anaphylactic response, including death which restricts multiple treatments with streptokinase (Collen, 1990; Jennings, 1996).

Although t-PA and urokinase are still widely used in thrombolytic therapy today, their expensive prices and undesirable side effects, such as risk for internal hemorrhage within the intestinal tract, their use is often limited and investigations are being pursued to search for cheaper and safer resources. Hence, the proposed research seems to be rationale to search for newer protease of therapeutic interest from bacterial strains.

METHODS AND MATERIALS

Mutant strain of *Bacillus licheniformis MZK05M9* was cultured in modified urea-glucose medium at 37°C under shake culture conditions for 48 h and 150 rpm. The culture supernatant obtained after centrifugation at 6,000 rpm for 10 min was subjected to ammonium sulfate precipitation. The salt precipitated enzyme was then concentrated by ultrafiltration through Centricon-100 centrifugal filter tube (Amicon Ultra) applying 5000 g centrifugal force for 15 min. The M.W. of purified protein was determined by SDS-PAGE analysis, taking protein sample from 100 kDa MWCO centricon tube retained enzyme, 100 kDa MWCO centricon tube permeate enzyme and 30 kDa MWCO centricon tube permeate enzyme.

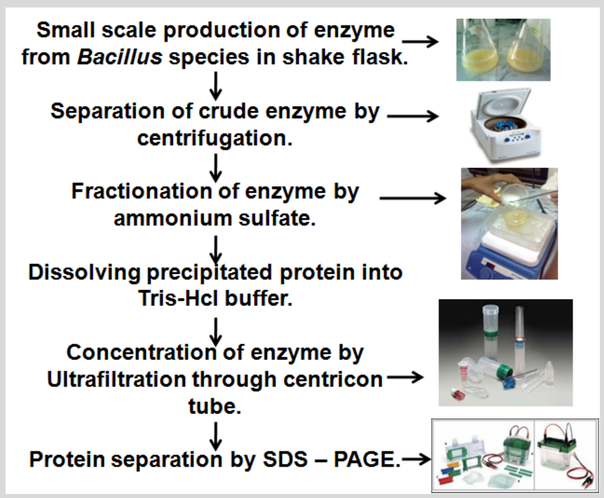

Figure 1 : Brief purification scheme

METHODS AND MATERIALS

The thrombolytic activity of permeate enzyme in terms of *in vitro* clot lysis assay was carried as reported earlier (Prasad *et al*., 2006).

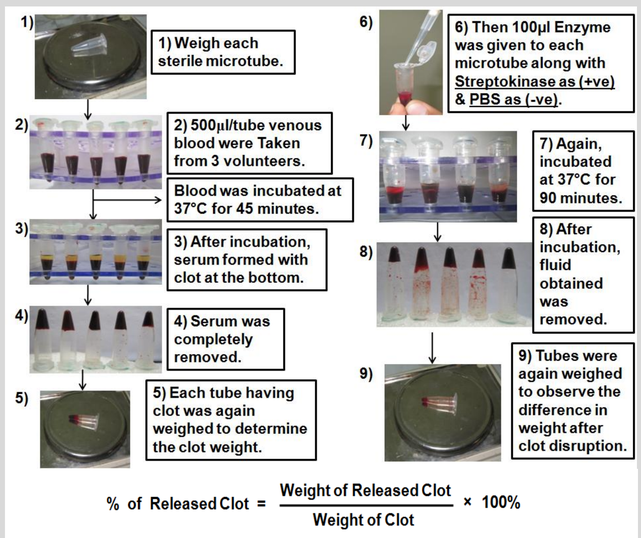

Figure 2 : *In-Vitro* Clot Lysis Assay Procedure

RESULTS

Purificaton step	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification fold	Recovery (%)
Crude enzyme	222030	264.286	840.112	1	100
Ammonium sulfate precipitated enzyme	65629.8	28.696	2287.071	2.722	29.559
100 kd Centricon tube permeate	22213.5	0.589	37713.922	16.49	10.00

Table 1 : Summary of partial purification steps of enzyme

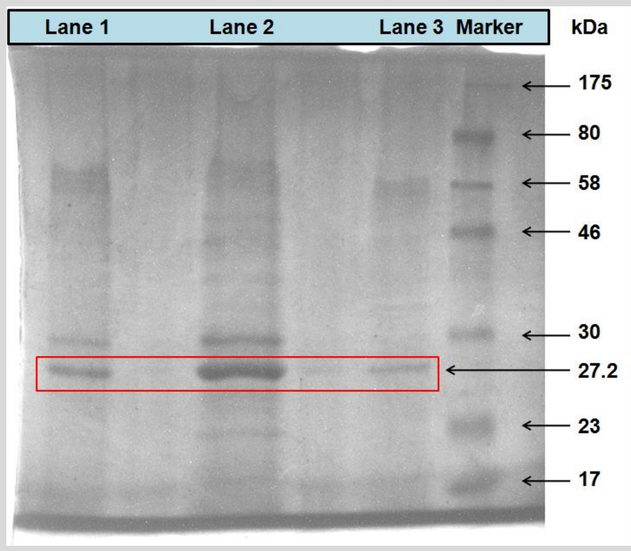

Figure 3 : SDS-PAGE of partial purified enzyme from *MZK05M9*.
Lane – 1 : 100kd MWCO Centricon tube retained enzyme,
Lane – 2 : 100kDa Centricon tube permeate enzyme,
Lane – 3 : 30kd MWCO Centricon tube permeate enzyme.

RESULTS

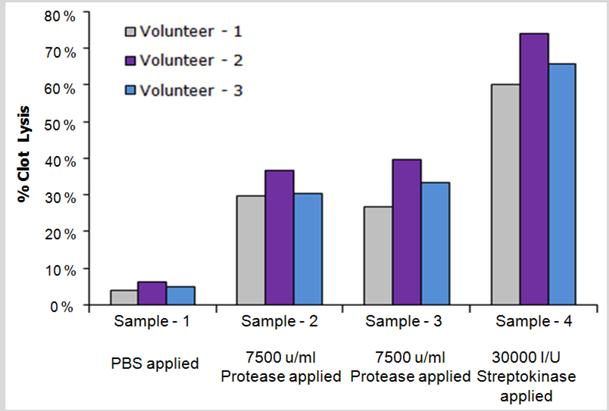

Chart 1 : Comparison between the volunteers in terms of thrombolytic effects of protease from *Bacillus sp. MZK05M9* with streptokinase and PBS.

DISCUSSION

The production method yielded 840.112 units/mg of the crude enzyme from *Bacillus sp.* strain *MZK05M9* and after partial purification it was 37713.92 units/mg with a purification fold of 16.5 and a recovery of 10%. These results suggests that our approaches for purification which includes ultrafiltration through centricon tube of specific MWCO to retain the specific molecular weight proteins was effective. Gel imaging analysis by Alphaview software predicted the presence of approximately 27.2 kDa protein band present in both protein sample taken from strain *MZK05M9.*

CONCLUSIONS

The search for alternative and complimentary therapy is still continuing due to some reasons including availability and diversity of natural resources, easy access and affordability. The nature of thrombolytic activity of the protease on blood clot was similar to that of streptokinase. From the above study, we can conclude that, the thrombolytic proteases obtained from *B. licheniformis* strain *MZK05M9* has the potential to be developed as novel and economic therapeutic agents for the treatment of thrombolytic and related diseases with less side effects in future.

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