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IDENTIFICATION OF A NOVEL SOURCE FOR PRODUCTION OF ANTITHROMBOTIC AND FIBRINOLYTIC AGENT AND DETERMINATION OF ITS EFFICACY IN *IN VITRO* AND *IN VIVO* MODEL FOR THROMBOLYTIC THERAPY

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ABSTRACT

In the present study two novel fibrinolytic microbes were identified and characterized. The preliminary screening, 16s rDNA and ITS sequence analysis proved that the strain S1PIMS belongs to Shewanella and F1PIMS belongs to Paecilomyces Species. The two organism exhibited strong proteolytic activities in plate assay. A suitable production media was optimized with different additives, carbon and nitrogen sources. The maximum proteolytic enzyme activities (80U/ml, 240U/ml) were achieved with media containing wheat bran and maltose. The enzyme production was found to be maximum at 60 hours of incubation with of 2% and 3%inoculum size. The effect of carbon sources from monosaccharides to polysaccharides, temperature, pH on the enzyme activity was also investigated at different level. Among the carbon sources, glucose (1% W/V), temperature at 40°C and pH 8 was found to be most effective. Partial purification of the enzyme was carried out by ammonium sulphate precipitation method and investigated for thrombolytic and fibrinolytic potential using widely used *in vitro* and *in vivo* model. The dialysed sample showed the halo zone (16.3 mm, 20.8 mm) on fibrin plate indicating enzyme has plasmin like activity. The enzyme also exhibited strong clot lyticpotentialin *in vitro* coagulation model. Significant reduction of tail thrombus length(2.6 cm, 1.8 cm) compared to negative control in λ- Carrageenan induced mouse thrombosis model clearly signifies the thrombolytic potential of the enzyme. The above optimistic result

established through our investigation will be useful towards development of an economical and safer thrombolytic agent for Cardio vascular diseases therapy.

Keywords: Fibrinolytic protease, Thrombolytic therapy, Partial purification, Clot lysis

INTRODUCTION

Cardio vascular diseases (CVD) are the most prevalent cause of death and disability around the globe. Though there has been revolutionary progress in understanding of cardiovascular conditions still morbidity and mortality due to CVDs are increasing persistently. Cardiovascular diseases include disorders of the heart and blood vessels includes coronary heart disease (heart attacks), cerebrovascular diseases (stroke), raised blood pressure (hypertension), rheumatic heart disease, congenital heart disease and diseases of arteries and arterioles. These diseases occur in children, infants and adults of both sexes. In addition to this, improper management of CVDs leads long term disability. According to World Health Organization (WHO), nearly17.5 million deaths from CVDs in 2005, which represents thirty percent of all global deaths and predicted 20 million deaths by 2015 due to CVDs[1].The risk factors for CVDs include behavioral factors such as smoking, deficient physical activity, unhealthy eating habit and too much alcohol consumption. A number of other risk factors with chronic diseases such as diabetes and kidney disease also add to CVDs. The exact mechanism and interactions between the risk factors and CVDs are not well understood. The above mentioned potential risk factors account for 90% of myocardial infarction and stroke, in all major regions of the world. Therefore, reducing the risk of CVD requires strategies that can be applied to large segments of the population. Current prevention strategies are limited due to lack of implementation of proven therapies, high cost and low affordability and fear of use of drugs and its adverse effect[2]. Intravascular thrombosis due to formation of blood clot (thrombus) in arteries is one of the main causes of CVDs. Several circulatory disorders such as myocardial, cerebral infarction, pulmonary emboli, deep vein thrombosis, strokes and heart attacks are mainly responsible for life threatening health problems among public. Surprisingly most affected individuals have very little or no awareness regarding thrombolytic disorders and its consequences. Therefore, the actual cases of incidence are still higher as these diseases are often unnoticed and undiagnosed until the onset of severe health problem.

The increase in expensive diagnostic and therapeutic procedure is another challenge in managing CVDs. A number of sources being exploited in search of compounds having anticoagulant activity. Many thrombolytic agents from different source also been identified and characterized so farwhich include snake venom, insect, earthworm, mushrooms[3,4]. However, affordability and adverse effect is the major concern to scientist as well as public. The cost of these therapeutic agents is generally high due to lack of cheaper source and expensive purification process.Therefore, Novel approaches for prevention are needed which involves wider use of proven, inexpensive and safe pharmacological therapies. Microbial therapeutic agent has attracted attention of research community as an alternative to other sources. In view of the above issues the present investigation is designed to find out and characterise an efficient microbial source for production of thrombolytic and fibrinolytic agent, optimization of various fermentation parameters and further determination of its efficacy using *in vitro* and *in vivo* model.

MATERIALS AND METHODS

Collection of material

Soil and water samples were collected from mangrove surface as well as 15-20 cm depth after removing the earth surface. The samples were diluted by making ten-fold serial dilution and the organisms were grown by enrichment techniques. Pure cultures were developed through repeated subculture and stored at 4°C until further use.

Isolation of proteolytic enzyme producing bacterial and fungal strain

For protease screening a loop full of isolated culture was streaked on to skimmed milk agar medium (1%)Hi-Media, Mumbai, India and the plateswere incubated at 37°C overnight. The isolates exhibiting distinct zone of hydrolysis were selected for further fibrinolytic screening.

Identification and characterization of selected organism

The morphological and biochemical characteristic of selected isolates were carried out according to[5]. Gram staining and different biochemical test such as Indole, Citrate utilization tests Voges Proskauer, starch hydrolysis, catalase test, H2S production test, Urease test and Carbohydrate fermentation of various sugars were studied. Molecular characterisation by 16s rDNA and ITS sequence analysis is considered to be most reliable method and widely used for phylogenetic analysis. Therefore, further confirmation of the strains that achieved the best enzyme production were sent for 16

rDNA and ITS sequencing to Genomebio technology, Pune. Identification was achieved by comparing the sequence data obtained with the reference data collection available in the public data base (nr) using BLASTN 2.3.1 program.The unrooted phylogenetic tree was generated using clustering algorithm Neighbor-Joining (NJ) method with 1000 bootstrap replicates, partial deletion (95% site coverage as cut off) using Molecular Evolutionary Genetics Analysis version 7.0 for bigdatasets MEGA7[6].

Fibrinolytic protease enzyme production

Overnight grown culture of bacterial inoculumwas added to the production media containing (g/100ml) Glucose 1g; peptone 0.5 g; NaCl 0.5g; Yeast extract 0.05g; $MgCl_2$ 0.085g; $CaCl_2$ 0.01g; $MnCl_2$ 0.001g (pH 7) and incubated at 37°C in a shaker incubator (120 rpm) for 48 hours. Effect of various carbon sources like glucose, fructose, sucrose (1%) and media additives like wheat bran, soybean meal, green gram powder (1%, 2%) were investigated for maximum enzyme production. At the end of incubation period the fermentation broth was centrifuged at 8000 rpm at 4°C for 15 minutes. The cell free supernatant was collected and used for protease enzyme essay. Similarly, the spores of fungal isolate were used to raise inoculum and inoculumwas introduced in to the production media (g/l) containingSucrose-30.0g; Sodium nitrate-2.0g; K2HPO4-1.0g; MgSO4.7H2O-0.5g; KCl-0.5g; FeSO4-0.01gand kept in shaker incubator at 120 rpm.Mycelia from the production media were removed and the filtrate was used for protease enzyme assay after the incubation period.

Optimization studies of fermentation parameters using one-factor-at-a-time

Several fermentation parameters such as effect of inoculum size (1 to 6%), initial pH (5-9) of the media, incubation time (20-100 hours) different temperature (30- 60°C) on production of fibrinolytic enzyme were investigated. The effect of various carbon sources such as glucose, fructose, sucrose and other media additives like wheat bran, soybean meal, rice bran, green gram husk (1% W/V, 2% W/V) were used to achieve the maximum enzyme production.

Protease assay

After the incubation period bacterial or fungal broth was centrifuged at 10000 rpm for 10 minutes at 4°C to obtain cell free supernatant. Protease activity was determined using casein as substrate[7]. Casein solution of 2% was prepared in 20 mM phosphate buffer. The buffer was gently heated with casein and left at room temperature until the

formation of uniform suspension of casein. A mixture of casein solution (0.5 ml) and crude enzyme solution of (0.5 ml) were incubate in water bath (55°C) for 10 mins. After incubation period the reaction was terminated by adding one ml of TCA (10%) and kept at room temperature for 10 mins. The reaction mixture was centrifuged at 10000 rpm for 10 mins and the supernatant was taken in a fresh tube. To the supernatant 5 ml of 0.44 M Na_2Co_3 and 1 ml of 2 fold diluted FC reagent was added. was added separate the unreacted casein. The resulting mixture was incubated for 20 mins at room temperature in dark. The absorbance was taken at 660 nm. A blank was prepared without enzyme solution. The enzyme activity was determined using standard tyrosine curve. One unit of protease is defined as the amount of enzyme that hydrolyses substrate casein and releases 1 µg of tyrosine per ml per minute under the standard assay conditions.

The enzyme activity was calculated with the following formula

$$\text{Unit/ml enzyme} = \frac{\mu \text{ mol Tyrosin eqvelent released} \times \text{Total Vol of assay}}{\text{Vol of enzyme used} \times \text{Length of assay} \times \text{Vol used in colorimetric determination}}$$

Partial purification of fibrinolytic enzyme

Ammonium sulphate method was used for partial purification of proteolytic enzyme from the production medium [8, 9]. The cell free supernatant (100 ml) was taken and ammonium sulphate was added gradually from 30% to 80% saturation with continuous stirring on a magnetic stirrer. The mixture was kept overnight at 4°C and centrifuged at 10000 rpm for 10 minutes. The precipitate was collected and dissolved in 50mM trisHCl buffer (pH7.2).

Dialysis

The dialysis membrane was activated by treating with water bath at 60°C for 10 minutes and washed with distilled water. A knot was made at one end of the dialysis membrane and sample was filled. The sample was dialysed against 10mM trisHCl buffer (PH 7.2) at 4°C. The buffer was replaced with the fresh one repeatedly in every hour for complete removal of salts.

Assay of fibrinolytic activity

Fibrinolytic activity was performed by plasminogen free fibrin plate assay [10, 11]. The fibrin plate consisted of fibrinogen solution (2ml of 2.5% W/V fibrinogen in 0.05 M PBS buffer, pH7.4). One ml of thrombin solution and 20 ml of agarose (1%) solution were plated. The plates were heated at 85°C for 30 minutes to deactivate the other fibrinolytic factors. Wells of 5mm diameter were punched using sterile cork borer. 40µl of partially purified enzyme solution were

loaded in to each well and incubated at 37°C overnight. The fibrinolytic activity was estimated by measuring the diameter of the zone of clearance on the fibrin plate. Streptokinase(30,000 IU) was used as positive control and phosphate buffer (20 mM) as blank.

***In vitro* lysis of blood clot**

In vitro lysis of blood clot was performed according to[12].Blood was collected by pricking ring finger and clotted artificially in glass capillary tubes. The fully loaded blood capillary was kept at room temperature for one hour to form firm blood clot. The capillary containing blood clot was cut in to pieces. The capillary after the formation of clot were placed in a petridish containing the enzyme solution 2 ml were shaken gently at frequent interval. Normal saline was used as control. The petridishes were kept at room temperature overnight.

***In vitro* thrombolysis test**

The experiment for thrombolysis was carried out according to the method described by[13, 14]. The whole blood from healthy individual was collected without the history of anticoagulant therapy. Each treatment of the experiment was repeated in three tubes. The venous blood drawn from healthy volunteers were distributed (500µl/tube) in apre-weighedependorf tube of 1.5 ml. The filled centrifuge tube was labeled and incubated at 37°C for 45 minutes for complete clot formation. Theserum was removed carefully without disturbing the blood clot. Each tube containing clot was weighed separately and the clot weight was determined individually (Clot weight= Wt of the clot containing tube- Wt of the empty tube). To each clot containing tube 100µl, 200µl and 400µl of enzyme solutions were added. All the tubes are now incubated at 37°C for 90 minutes and clot lysis was observed. Streptokinase and distilled water was used as positive and negative control. After the incubation period the released fluid was aspirated using a syringe without disturbing the clot and the tubes were weighed again. The difference in weight taken before and after clot lysis was expressed as percentage of clot lysis.

$$\textbf{\% Clot lysis} = \frac{\textbf{Wt of the clot after lysis}}{\textbf{Wt of the clot before lysis}} \times \mathbf{100}$$

***In vivo* study**

Adult Wister Albino rats (200-250g) of both sexes were chosen for the experiment. Animal handling procedures were strictly followed throughout the experiment according to the international guidelines. All animals were fasted 24 hours prior of starting the experiment and had free access to water. Animals were divided in to three groups with four animals in each group.

Acute toxicity study

Experimental protocol for toxicity study were followed according to our previously published report [15]. The toxic effects of the partially purified enzyme were evaluated before using for further experimentation according to the method described by Wang, 2008. The animals were acclimatized to in house condition before starting the experiment. The protocols were followed according to internationally accepted OECD-423 guidelines throughout the experiment. Subsequently normal saline to the control group and dose of partially purified enzymes (0.2ml, 0.5ml) to the experimental group were administered orally. All animals were fasted 4 hours prior to treatment but had free access to water. After administration of the test drugs sign of toxicity and mortality was observed with special attention for the first four hours and daily four times thereafter for a period of fourteen days. Any symptoms of ill health or mortality were recorded.

Thrombolytic activity of partially purified enzyme on mouse tail thrombus model

The animals were divided in to four groups, the first group served as control and was treated with normal saline. The group 2 and 3 was treated with 0.2 and 0.5 ml of partially purified enzyme solution dissolve in normal saline by oral administration for one week. After the last treatment, along with the enzyme solution, λ- Carrageenan (20µl/10g BW) were injected subcutaneously to induce thrombus in tail. The length of tail thrombus was measured at 24 hrs of injection. Group 3 act as positive control treated with urokinase 0.5 ml (4 mg /kg BW). Each experiment was carried out at a minimum of three replicate.

RESULTS AND DISCUSSION

The samples were processed for enrichment with media for bacteria and fungi separately using serial dilution method. The sample contains an average of 12×10^4 CFU (colony forming unit) per gram of soil and 8×10^4 CFU (colony forming unit) per ml of water sample. Based on the highest proteolytic activity in term of zone of clearance on skimmed milk agar plate one strain from bacteria (S1PIMS) and one from fungi (F1PIMS) were selected for further study (Fig. 1).

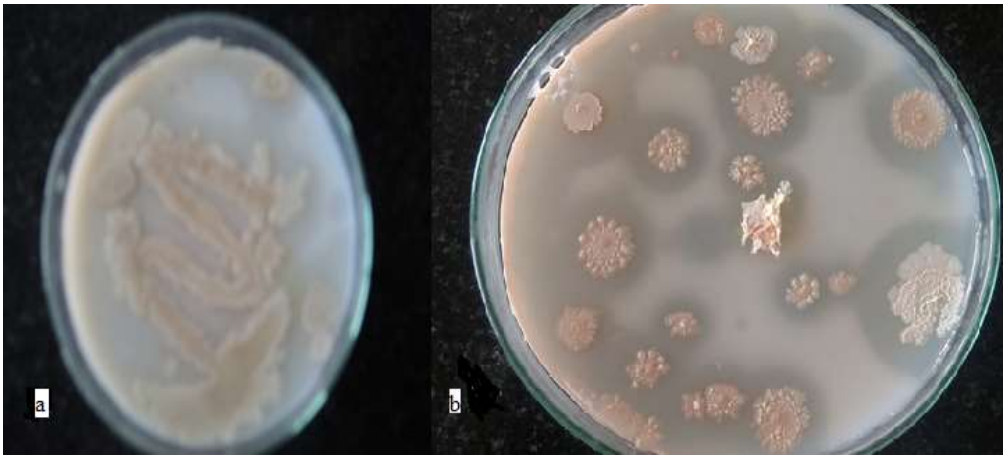


Fig. 1 Degradation of substrate by isolated organism (a) S1PIMS and (b) F1PIMS, showing clear Zone

Identification and characterization of bacterial isolates

S1PIMS was observed to be as gram-negative rods and showed positive for citrate and catalase whereas indole, methyl red, Voges–Proskauer, Triple Sugar Iron, urease, oxidase and nitrate reduction test were found to be negative.For furthercofirmation the 16s rDNA from S1PIMS and ITS from F1PIMS was sequenced. The resulting sequence was used for homology search and deposited in GenBank database with accession no KX523175 and KX523176. The result of BLASTn analysis showed that 16s rDNA of the bacterial strain S1PIMS has gene sequence closely related to Shewanella sp. (100%) while the ITS sequence of fungal strain F1PIMS exhibited 100% similarity to Paecilomyces sp. To determine the evolutionary relationship,phylogenetic tree was constructed usingclustering algorithm Neighbor-Joining(NJ) method with 1000 bootstrap values (Fig. 2and Fig. 3). The result of the phylogenetic classification wasfound to be consistent and therefore it was reconfirmed that strain S1PIMS belongs to Shewanella sp. and strain F1PIMS belongs to Paecilomyces sp.

Optimization of fermentation parameters

Both organisms were tested for proteolytic potential and extracellular protease production through submerged fermentation. Production media supplemented with different additives, carbon and nitrogen sources were used in order to achieve high enzyme activities.Carbon sources such as glucose, fructose, sucrose and media additives like wheat bran, soybean meal, rice bran, green gram powder were tried. After 60 hours of incubation isolate S1PIMS achieved 80U/ml and F1PIMS achieved 240U/ml of

proteolytic enzyme (Fig. 4).It is very important to find out the incubation time at which the organism exhibits highest enzyme production [16]. The protease production was maximum at 60 hours of incubation and gradual decrease in activity from 60 to 100 hours was observed. The current finding is supported by [17],who also reported the maximum protease production during 60 hours of incubation by Bacillus Sp.The production of protease was carried out with different inoculum size ranging from 1 to 6%. However, it was noticed that inoculum size at 2% shows the highestactivity in S1PIMS whereas the isolate F1PIMS showed maximum activity at 3% inoculum size (Fig. 5). The enzyme activity was gradually decreased thereafter. Similar results were also reported by[18].

As the pH of the production medium strongly influence the enzymatic activity, the optimum pH profile of the isolates was studied at varying pH values. Interestingly, the protease enzyme was found to be most active within the pH range of 7.5 to 8.5 with an optimum pH at 8. However, it was observed that there was decreased in enzyme production beyond pH8.5 (Fig. 6).Similarly,the effect of temperature on the enzyme activity was also investigated at different level. The crude enzyme of isolate S1PIMS was active within the range of 35 to 50°C. Gradual increase in the enzyme activity was observed with increase in the temperature. The optimum temperature for maximum enzyme production was 40°C. However, increase in temperature beyond 40°C leads to decrease in enzyme production which indicates temperature plays key role in protease production (Fig. 7). The yield of low enzyme at high temperature may be due to change in physiological condition. Thehighest protease production at 37°C by Bacillus species was reported by [19]. Our result complies with [20] who also reported the optimum temperature of 40°C for protease production in *Bacillus brevis*.

The effect of carbon sources from monosaccharides to polysaccharides could influence the enzyme activity [20, 21].Studies on the effect of carbon sources revealed that glucose was the most preferred carbon source compared to fructose and sucrose. Maximum protease production was observed when the medium supplemented with glucose (1% W/V) along with wheat bran powder (1% W/V) in case of isolate S1PIMS (80 U/ml). Where as in case of isolate F1PIMS among all the media additives used, the maximum protease production was achieved with glucose and 2 percent green gram husk (240 U/ml)(Fig. 8).

The wheat bran at 2% level was proved the second most preferred additives for protease production in case of bacterial isolates. It is reported by many researchers that the agricultural byproducts contain protein, carbohydrates, fats, minerals, vitamins and amino acids which is essential for microorganism for its growth and biosynthesis of enzymes [23].Several other authors also reported the agricultural byproduct in production medium helps in enhancing the enzyme production and activities[24,25,26]. Wheat bran contains 8.5% of starch which becomes saccharified to glucose due to the action of enzyme during the course of fermentation[27].

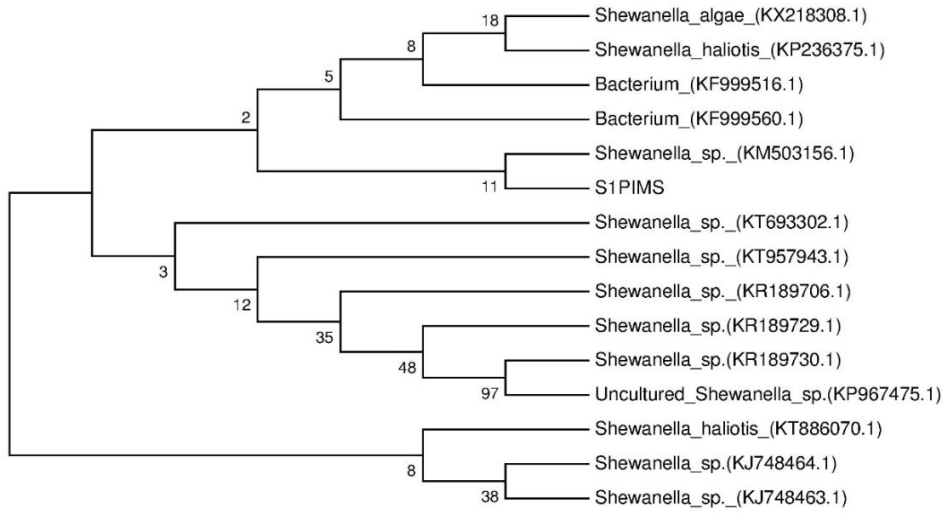


Fig. 2Phylogenetic tree based on the 16S rDNA sequence of Shiwanella sp. KX523175, Numbers in parenthesis are Gen bank accession numbers of published sequences

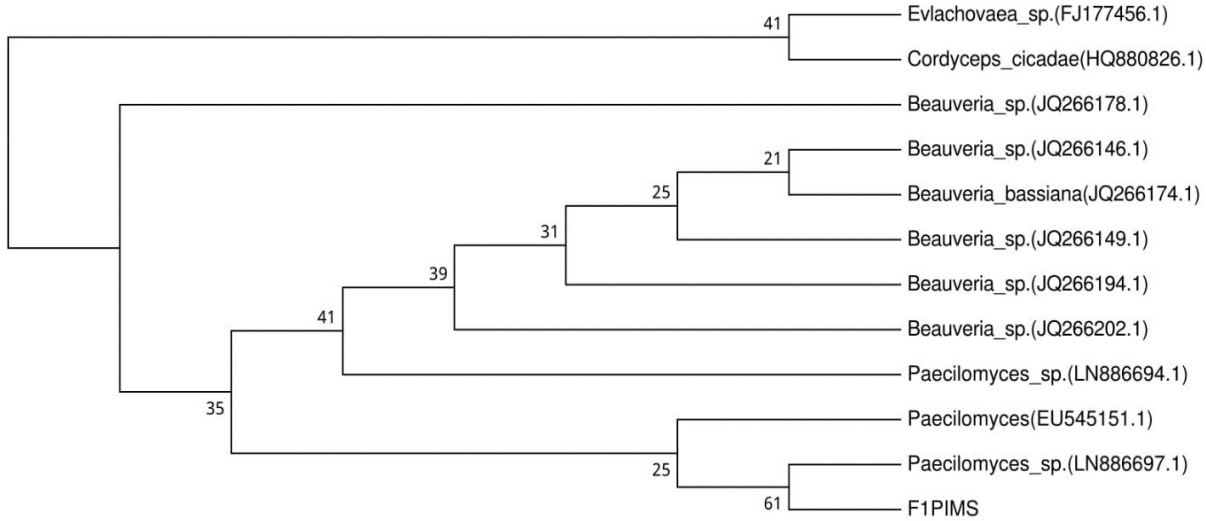


Fig. 3 Phylogenetic tree based on the ITS sequence of Paecilomyces sp. KX523176, Numbers in parenthesis are Gen bank accession numbers of published sequences

Partial purification of Fibrinolytic protease

The produced protease from cell free filtrate was partially purified by widely used ammonium sulphate precipitation method up to (30%, 40%, 50%, 60%, 70%, 80%) saturation. Among all the used concentration, ammonium sulphate at concentration of 60% showed highest precipitation. The precipitated fraction of 60% ammonium sulphate was subjected to dialysis for removal of excess mineral salts. The specific activity of dialysed fraction were tested and observed to be 272 U/ml and 864 U/ml respectively in case of S1PIMS and F1PIMS(Fig. 9). Thus thedialysed sample showed 3.4 and 3.6fold increase in the specific activity as compared to the crude supernatant. Similarresult of increase in specific activity by ammonium sulphate precipitation at 60% and 70% saturation by [28]. Now the dialysed sample referred as partially purified enzyme and further experimentation was carried out for fibrinolytic potential using the same sample. The ammonium sulphate method of precipitation is the most frequently used method in laboratory and industry and does not cause the denaturation of protein with maximum enzyme purification[29, 30, 31, 32]

The dialysed sample showed the zone of clearance (16.3 mm, 20.8 mm) surrounding the well. As the enzyme formed a clear zone on the plasmin free fibrin plate which indicates the partially purified enzyme has plasmin like activity that can degrade fibrin clot by fibrinolytic activities. It was observed that the dimension of the halo zone was increased with gradual increase in the concentration of enzyme added to the well. In the plasminogen free fibrin plate the fibrinolytic activity of partially purified enzyme was comparable to that of urokinase in term of clear zone (23.6 mm). Since the diameter of clear zone on fibrin plate is directly proportional to the activity hence it can be concluded that the enzyme purified from isolate S1PIMS and F1PIMS have strong fibrinolytic potential. The fibrinolytic activity by fibrin plate assay method was followed by many authors [33, 34].Fibrinolytic activity from halophilic bacteria in plasminogen free fibrin plate[11]. The strong fibrinolytic potential of the purified enzyme may serve as candidate for thrombolytic therapy.

***In vitro* clot lysis**

The thrombolytic activity was also studied using *in vitro* coagulation model by inducing clot of human blood within glass capillary. The partially purified enzyme

exhibited strong activity in dissolving blood clot. The petriplate containing enzyme solution lysed the clot formed inside the capillary, whereas no visible changes were observed in the plate containing normal saline (Fig. 10). The above result further confirms clot lytic potential of the purified enzyme. However, it was observed that decrease in antithrombotic activity with increase in blood clotting time. Our result is in agreement with [35],who stated that thrombin promotes the coagulation pathway with increase in time duration. It was also noticed that the consistency of blood clot within the capillary was reduced with increasing concentration of sample. We noticed that the enzymes purified from isolate S1PIMS and F1PIMS exhibited thrombolytic activity as low as 20µl. Activity at such low concentration is especially useful in preparation of commercial formulation.

***In vitro* thrombolytic test**

Thrombolytic activities of the partially purified enzymes were evaluated using thrombolytic model. The thrombolytic activity assessment showed the highest activity with increasing concentration(Table 1). Among the two isolates the fungal isolate F1PIMS showed the highest percent of clot lysis. In general, all the tested concentration exhibited thrombolytic activity ranging from 42.44 to 60.86 percent.The maximum average thrombolytic activity of 46.21% and 56.49 was shown by S1PIMS and F1PIMS respectively after 90 minutes of incubation. The positive control (streptokinase) after incubation of 90 minutes at 37°C showed highest clot lysis 79.90% on average.

Table 1 Effect of different enzymes on *in vitro* clot lysis

Name of the isolate	Concentration of enzyme solution (µl)	% of clot lysis
S1PIMS	100	42.44
	200	46.62
	400	49.59
F1PIMS	100	52.91
	200	55.72
	400	60.86
Negative control	100	3.06
	200	3.09
	400	3.05
Positive control	100	74.66
	200	78.61
	400	86.45

From the above result it can be concluded that the organism produces the enzyme which has ability to disrupt the clot efficiently and can be used as thrombolytic agent for treatment of acute mayocardial infarction, deep vein thrombosys, pulmonary

embolism, acute ischemic stroke etc. However further studies on quantification, cloning and characterization of enzyme, *in vivo* toxicity study is necessary for utilizing as effective drug.

Thrombolytic activity using mouse thrombosis model

From the acute toxicity study conducted for partially purified enzyme there was no mortality or any behavioral changes were observed in test animals. Also, there was no obvious change in the body weight. λ- Carrageenan induced thrombosis model was used to test the thrombolytic activity of the partially purified enzyme. Tail thrombus was observed after 24 hours of λ- Carrageenan injection. The partially purified enzyme solution from S1PIMS and F1PIMS were subcutaneously injected and the length of the visible tail thrombus was measured. Significant inhibition of tail thrombus length was observed in λ- Carrageenan induced thrombosis model. The average thrombus length in group one was 4 cm where as in group two visible reduction of thrombus length of 2.6 cm, 1.8 cm respectively was observed with increasing dose of enzyme solution (0.2 ml, 0.5 ml). The thrombus was negligible in case of group three (positive control). Our result suggests that the enzyme solution can effectively prevent the thrombus by λ- Carrageenan induced thrombosis and work on dose dependent manner.

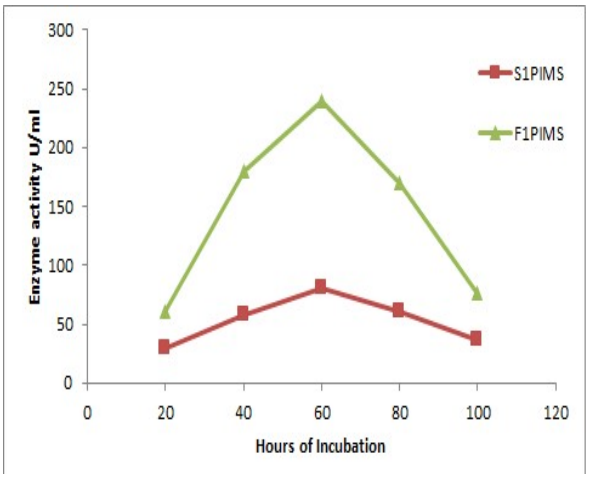


Fig. 4 Proteolytic activity of S1PIMS and F1PIMS

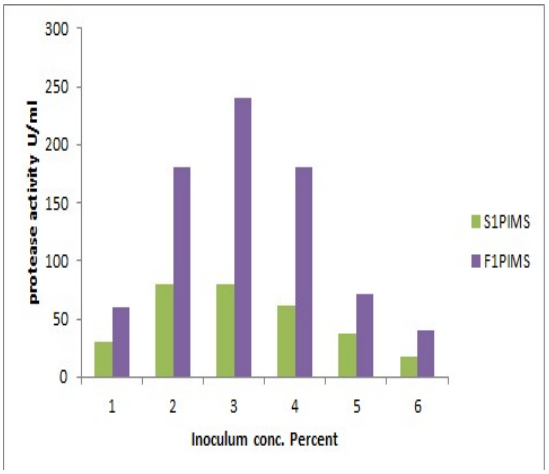
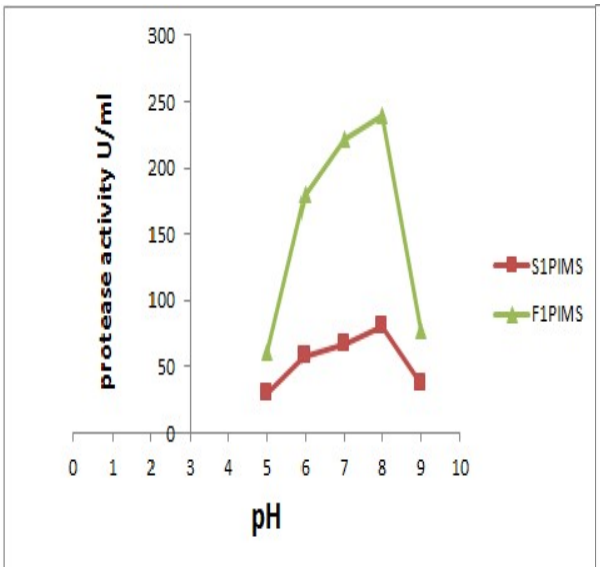


Fig. 5. Effect of inoculum concentration on protease production



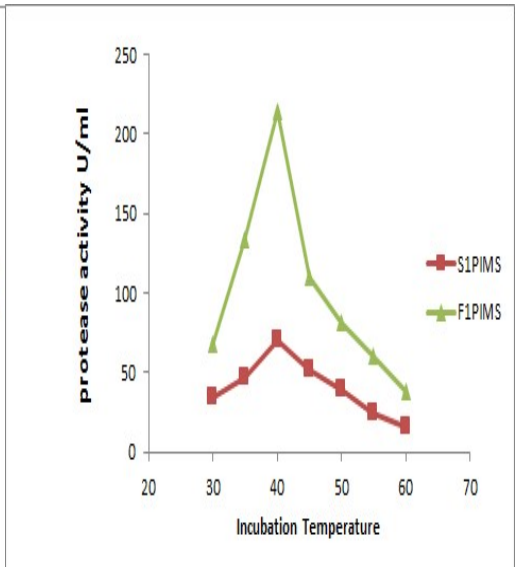


Fig. 6 Effect of pH on protease productionFig. 7 Effect of temperature on protease production

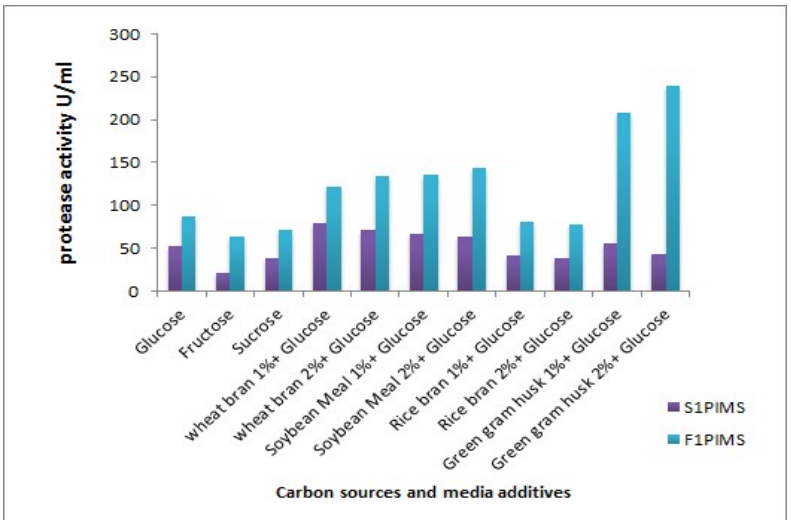


Fig. 8 Effect of carbon sources on protease production

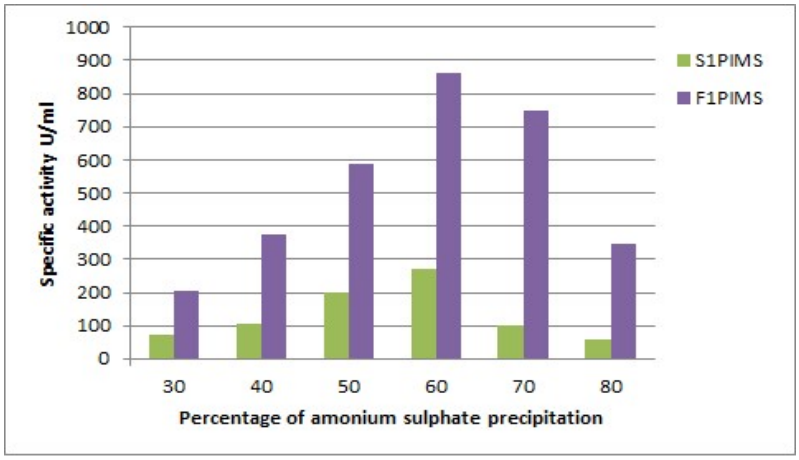


Fig. 9 Specific activity of dialysed sample at different percentage of ammonium sulphate Precipitation

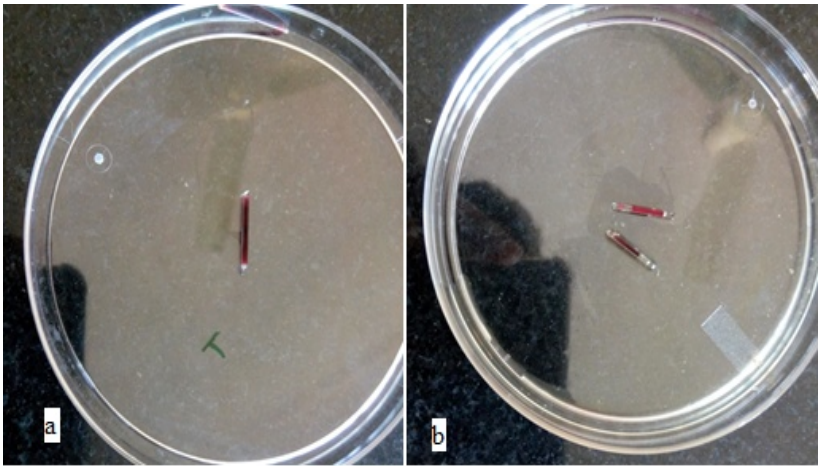


Fig. 10 (a)Artificial blood clot in glass capillary with Normal saline after incubation (b) Artificial blood clot in glass capillary with enzyme solution showing clot lysis after incubation period

CONCLUSION

From this research we could assume that the enzymes from S1PIMS and F1PIMS organism have similar mechanism of streptokinase. They exhibited platelet disaggregation, fibrinolytic and thrombolytic properties. Therefore, the present investigation holds great value with beneficial virtue on clot lysis, as efficient strain could add value to pharmaceutical industries. Screening and characterisation of efficient strain, optimization of production medium, various key parameters for fermentation were established through our present investigation. However further studies need to focused on purification, extensive characterization, Cloning and expression studies of the enzyme.

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