CHAPTER IV

RESULTS

1. Screening of Serine beta-fibrinogenase clones from cDNA Library

1.1 Plaque-lift hybridization

After hybridization with RVV141 -serine beta-fibrinogenase probe and washing out at low stringency condition. The hybridized membranes were detected with Phospho-Imager. The hybridized membranes not showed any signal as shown in **Figure 7**.

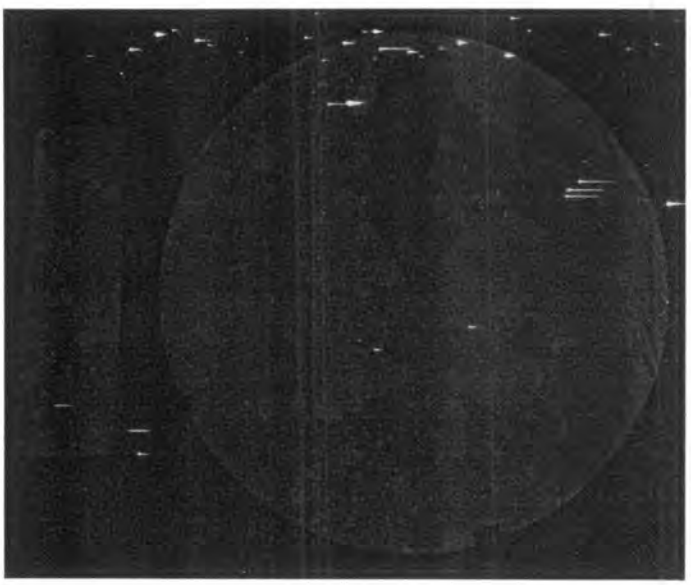

Figure 7 The hybridized membranes of serine beta-fibrinogenase clones in plaque lift hybridization. It's not shown signal.

1.2 Analysis of the RVV141 pBK-CMV phagemid clone containing partial serine beta-fibrinogenase gene

After detection of RVV141 serine beta-fibrinogenase clones on X-ray film, the X-ray films did not show positive signal in any plaques. From our previous studies, the RVV141 pBK-CMV double strand DNA phagemid were obtained from Expressed Sequence Tags (ESTs) study. And then RVV141 DNA phagemid was transformed to *E.coli* competent cell. DNA phagemid was extracted and double-digested with *Eco*R I and *Xho* I, identified fractionated on agarose gel electrophoresis the clones which contain of about 0.8 kb insertions as shown in **Figure 8**.

Figure 8 An ethidium bromide stained agarose gel of double strand phagemid of RVV141 pBK-CMV digested by *EcoR* I and *Xho* I. Lane M; 300 ng of 100 bp DNA ladder, lane 1; digested-RVV141 pBK-CMV containing 0.8 kb inserted cDNA.

1.3 Computational searching analysis of partial cDNA sequences

The RVV141 cDNA sequences of 799 nucleotides was obtained from DNA sequencing (**Figure 9**) and identified against GENBANK database using the BLAST N program as shown in **Figure 10**. The closest sequence of RVV141 was serine beta-fibrinogenase precursor from *Macrovipera lebetina* (VLBF, GENBANK accession number AF536235) with 91% nucleotide sequence identity. The RVV141 was identified as a truncated fragment.

Partail cDNA sequences of RVV141

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  1 TCTGGGGGACCCCTCATCTGTAATGGACAAATCCAGGGTATTGCATCTTGGGGGCACTAT
  1  S  G  G  P  L  I  C  N  G  Q  I  Q  G  I  A  S  W  G  H  Y
 61 CCTTGTGCCCAACCGCTTAAGCCTGGCCACTACACCAAGGTCTTTGATTATACTGATTGG
 21  P  C  A  Q  P  L  K  P  G  H  Y  T  K  V  F  D  Y  T  D  W
121 ATCCAGAGCATTATTGCAGGAAATACAAATGTGACTTGTCCCCCGTGAaaactttgaaa
 41  I  Q  S  I  I  A  G  N  T  N  V  T  C  P  P  *
181 aatttaagaggaggaaaatgtggcattagtacatctcttctatatccctaatcatattc
241 aagtgcattggaatatattcccaagcagtaagctttttaaagactcaaataggactgcct
301 ttggagtaagaaatgctcaaaatagtgctgcagggatcatgtcccatttaatttcagtat
361 aaaacaatcttagtaaaatgaaagcttgtcttacggtgcagtgcaaaattttctgactct
421 aaaatggaccattccaaatattttaatctctgaatatcttttcatttctctccacttctg
481 ggacagtgggatccttgatgctctctgagcttgttttcttgcagacatttcattacccag
541 ctagttaacattgtcagcgctagaatattcttttctattggtacttctgtggcatttgca
601 atacgttcatatggagtcatgcagtcatcctacaaccatatccatatacctgagtcccac
661 tgttgcttaaaaaggatgccagattaacccccacttcccaatcaatatattgaatctttt
721 gagaaccatgttttcatgtaaattctcaggtatccacagcaataaaatcgtataaatcgt
781 caaaaaaaaaaaaaaaa
```

Figure 9 Partial cDNA sequence of RVV141. Identical partially cDNA sequences. Stop codon was shown in box. The underlined is 3'UTR sequence.

Distance tree of results NEW

Legend for links to other resources: U UniGene E GEO G Gene S Structure M Map Viewer

Sequences producing significant alignments:
(Click headers to sort columns)

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
AF536235.1	Macrovipera lebetina serine beta-fibrinogenase precursor, mRNA, com	1061	1061	85%	0.0	91%	
D67082.1	Trimeresurus gramineus mRNA for serine protease, complete cds	1031	1031	84%	0.0	90%	
U31417.1	Trimeresurus mucrosquamatus preprotrimubin mRNA, complete cds	1026	1026	85%	0.0	90%	
D67080.1	Trimeresurus flavoviridis mRNA for serine protease, complete cds	1018	1018	84%	0.0	90%	
D67083.1	Trimeresurus gramineus mRNA for serine protease, complete cds	1011	1011	84%	0.0	90%	
AF336126.1	Gloydius ussuriensis thrombin-like serine protease mRNA, complete c	1000	1000	84%	0.0	89%	
AF370124.1	Gloydius ussuriensis thrombin-like enzyme (TLE) mRNA, complete cds	996	996	84%	0.0	89%	
D67079.1	Trimeresurus flavoviridis mRNA for serine protease, complete cds	987	987	84%	0.0	89%	
AY251282.1	Bothrops jararacussu serine protease mRNA, complete cds	977	977	84%	0.0	89%	
U32937.1	Agkistrodon ussuriensis calobin mRNA, complete cds	976	976	84%	0.0	89%	
AJ278786.1	Gloydius shedaoensis partial mRNA for defiberase	974	974	84%	0.0	89%	
D67084.1	Trimeresurus gramineus mRNA for serine protease, complete cds	968	968	84%	0.0	89%	
U21903.1	Trimeresurus steinegeri venom plasminogen activator precursor (TSV	955	955	85%	0.0	88%	
DQ396477.1	Lachesis muta serine protease precursor, mRNA, complete cds	937	937	84%	0.0	88%	
AF163973.1	Macrovipera lebetina factor V activating enzyme precursor, mRNA, co	922	922	85%	0.0	88%	
AB178322.1	Bothrops jararaca mRNA for hypothetical protein, complete cds, clone	894	894	97%	0.0	85%	
D67078.1	Trimeresurus flavoviridis mRNA for serine protease, complete cds	883	883	84%	0.0	87%	
AB031394.1	Bothrops jararaca mRNA for protease A, complete cds	872	872	84%	0.0	87%	
AB178321.1	Bothrops jararaca mRNA for hypothetical protein, complete cds, clone	867	867	84%	0.0	87%	
D67085.1	Trimeresurus gramineus mRNA for serine protease, complete cds	841	841	83%	0.0	86%	
AF018568.1	Gloydius ussuriensis capillary permeability-increasing enzyme-2 (CPI-	833	833	85%	0.0	86%	
AF490536.1	Bothrops insularis cluster BITS01A serine proteinase precursor, mRNA	815	815	84%	0.0	86%	
AB004067.1	Bothrops jararaca mRNA for KN-BJ2, complete cds	732	732	69%	0.0	87%	
L07308.1	Calloselasma rhodostoma ancrod mRNA, complete cds	621	621	84%	1e-174	82%	
D67081.1	Trimeresurus gramineus mRNA for serine protease, complete cds	569	787	78%	5e-159	89%	
AB178323.1	Bothrops jararaca mRNA for hypothetical protein, complete cds, clone	558	872	83%	1e-155	88%	
AF056033.1	Gloydius halys salmobin mRNA, complete cds	473	923	78%	4e-130	90%	

Figure 10 Homology searching of partial cDNA sequence RVV141 using BLAST N program.

2. Molecular cloning of Full length cDNA of serine beta-fibrinogenase homolog (RV SBF)

2.1 Obtaining cDNA encoding mature sequence of serine beta-fibrinogenase homolog (RV SBF)

The cDNA encoding mature sequences of serine beta-fibrinogenase homolog was amplified by reverse transcription-polymerase chain reaction (RT-PCR) using two primers. The degenerate forward primer, SBF-F was designed by aligned consensus more than 20 highest homology of RV SBF sequence that were designed from conserved sequences among the serine beta-fibrinogenase downstream the signaling sequence and reverse primer. SBF-R was designed from 3'end of coding sequence of serine beta-fibrinogenase homolog (RVV141), after that amplified by one step RT-PCR. The estimated PCR product size is 705 bp DNA in length (**Figure 11**). The PCR product was cloned and selected for 10 clones sequencing that was shown in **Figure 12**. Mature sequence of Serine beta-fibrinogenase showed highest homologous to *Macrovipera lebetina* serine beta-fibrinogenase precursor (VLBF) (GENBANK accession number AF536235) which found in *Macrovipera lebetina* venom (Levantine viper). An alignment showed 90% nucleotide sequence identity (**Figure 13**). Moreover positive 10 clone selected were aligned with CALUSTAL X that found all of sequence highly conserve in one pattern (**Figure 14**).

Figure 11 An ethidium bromide stained agarose gel of cDNA encoding mature sequence serine beta-fibrinogenase. Lane M; 300 ng of 100 bp DNA ladder, lane 1; 705 bp of cDNA encoding mature sequence serine beta-fibrinogenase.

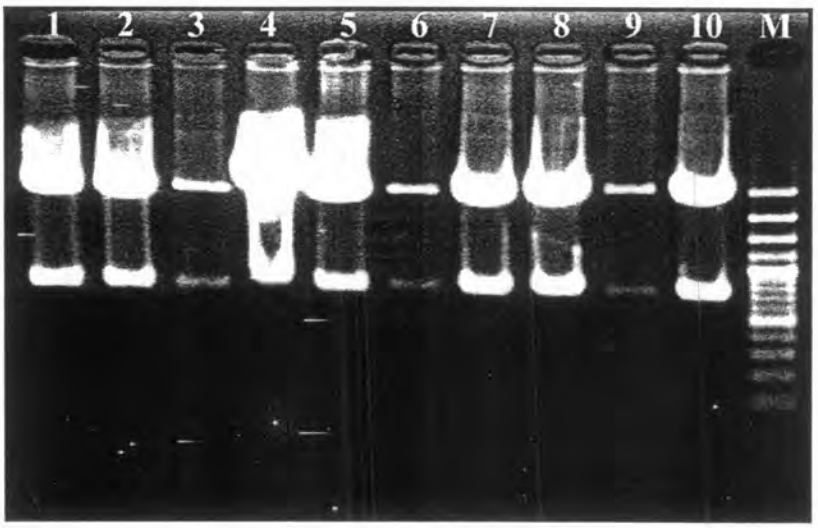


Figure 12 An ethidium bromide stained agarose gel showing *EcoR* I digested recombinant serine beta-fibrinogenase/pGEM®-T easy vector. Lane M; 100 bp DNA ladder, lane 1-10; digested serine beta-fibrinogenase in pGEM®-T easy vector.

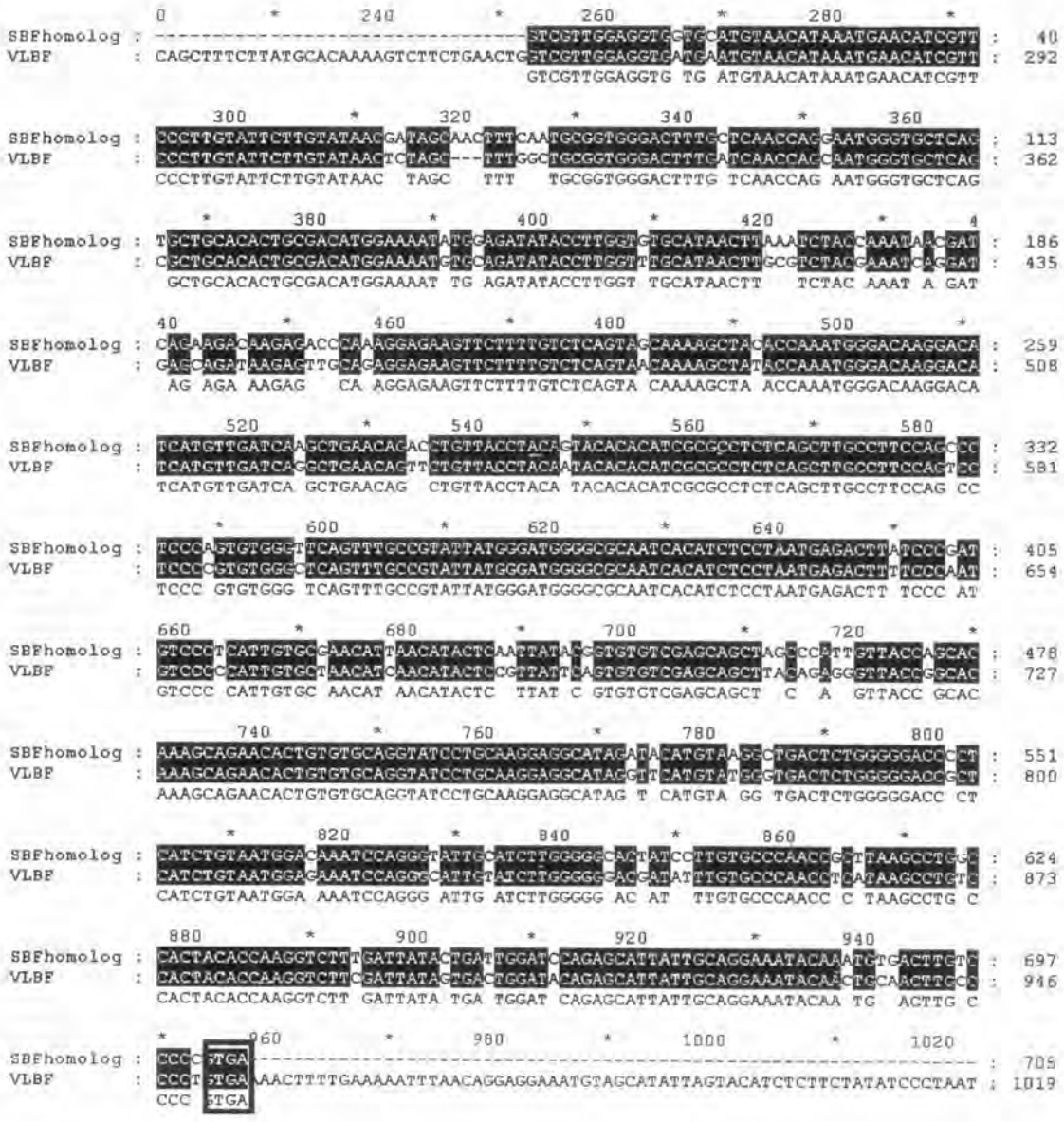


Figure 13 An alignment comparison of cDNA encoding mature sequences serine beta-fibrinogenase homolog and *Macrovipera lebetina* serine beta-fibrinogenase precursor using CLASTALX program. Stop codons were shown in box.

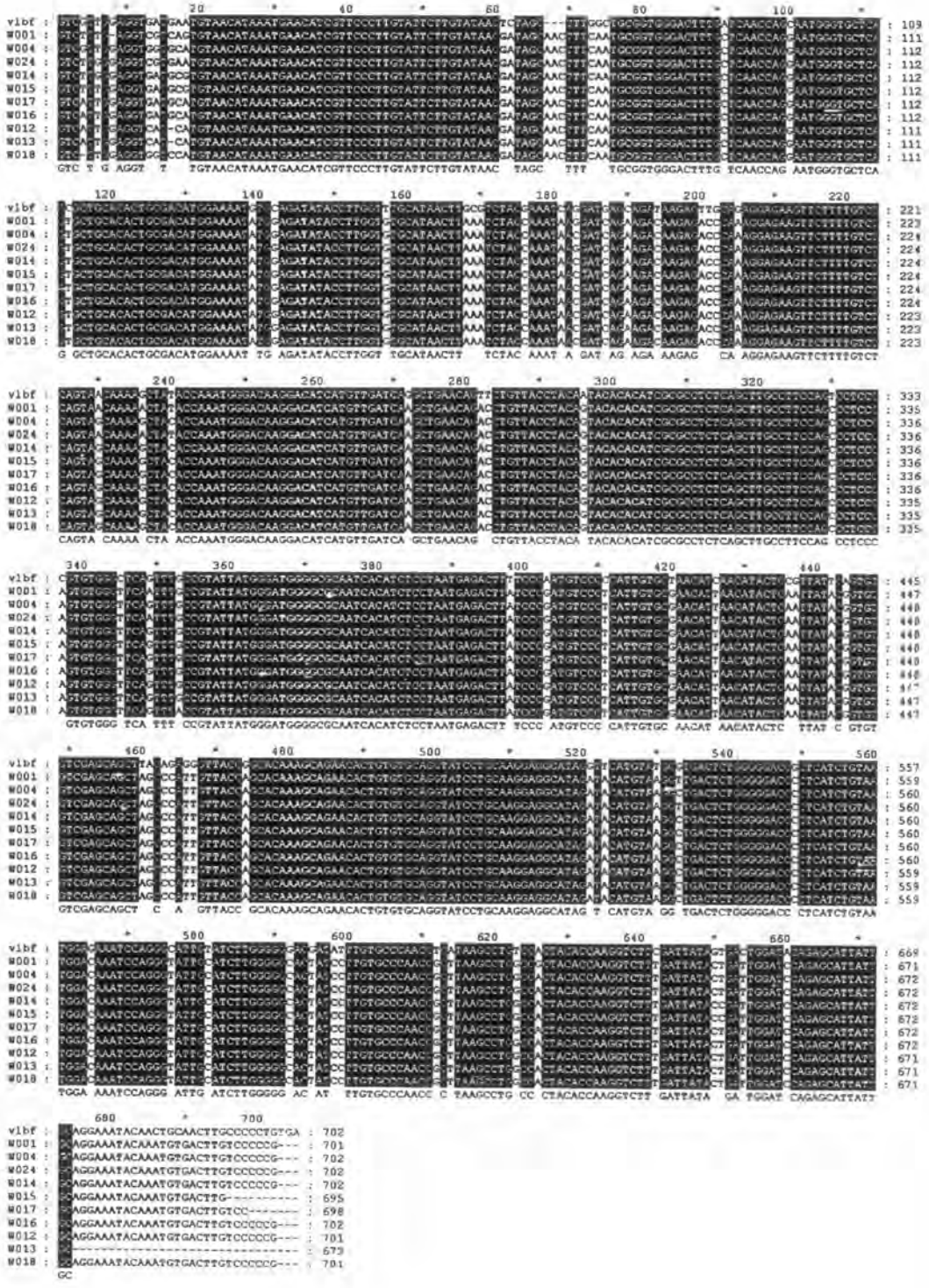


Figure 14 An alignment of 10 cDNA clones encoding mature sequences of RV SBF using CLASTAL X program.

2.2 Subcloning cDNA encoding mature sequence to Expression Vector

W001 and W004 clones were selected from 10 positive clones of pGEM® -T easy vector. The insert, 700 base pairs in size, was obtained by digestion with restriction enzymes (*BamH* I and *EcoR* I) prior to perform the ligation. After that, mature sequence was ligated into plasmid vector (the pTrc-HisA, pET32+ expression vector) and then transformed to *E. coli* competent cells. After recombinant clone screening by restriction enzyme analysis, both pTrc-HisA and pET32+ expression vector showed the mature cDNA inserts. Unfortunately, after DNA sequencing, there was one nucleotide deletion in 5'end of mature cDNA which will cause error in protein translation (**Figure 15**) in W001 clone. Therefore, W004 clone which contains the correct sequence at 5'end was used in the further experiment,

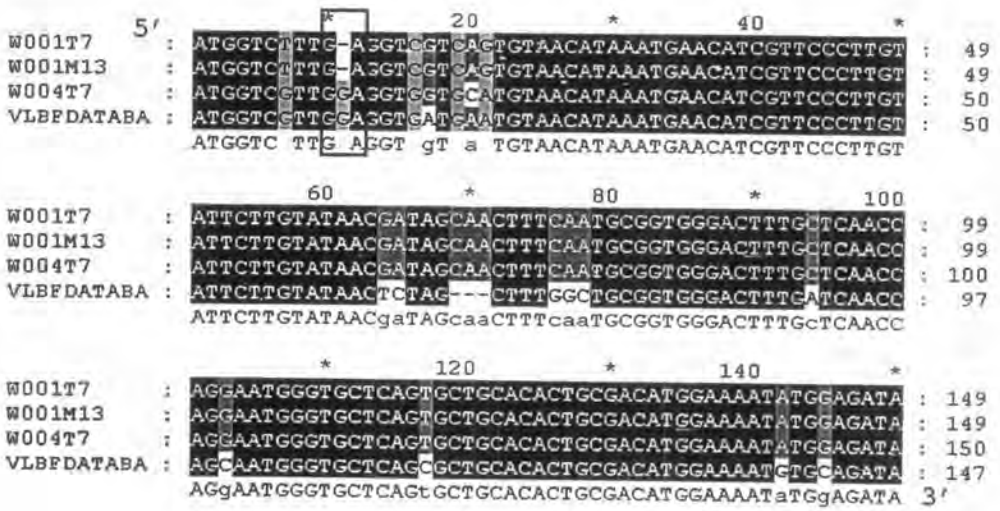


Figure 15 An alignment of selected mature cDNA clones of RV SBF from pGEM®-T easy cloning. W001; showed one nucleotide deletion at 5'end sequences, W004; the correct clone, VLBF; serine beta fibrinogenase from *Macrovipera lebetina*.

2.3 Obtaining Full length cDNA sequences of serine beta-fibrinogenase by 5'RACE

Mature cDNA sequences of RV SBF were used to design specific primers in 5'RACE to obtain the complete cDNAs encoding RV SBF. The 5' RACE products, about 550-600 base pairs in size (**Figure 16**), were eluted and subcloned into pGEM®-T easy vector and then transformed into *Escherichia coli* JM109 strain. The positive clones were identified by white colonies using blue-white color selection system. About 10 positive clones were identified and isolated by alkaline lysis minipreparation method and then digested with *EcoR* I to screen for clones that were contained estimate size insert 600 base pairs (**Figure 17**). After that, the plasmid containing inserts were sequenced using ABI PRISM (Perkin-Elmer) system. SP6 and T7 primers were used for sequencing the plasmid containing the expected insert. The sequence comparisons were analyzed with GENBANK database using BLAST N program. The results indicated that 5' RACE amplified products were highest homologous to *Macrovipera lebetina* serine beta-fibrinogenase precursor (VLBF). Furthermore, alignment of full length cDNA sequences serine beta-fibrinogenase homolog with database was also highest homologous to *Macrovipera lebetina* serine beta-fibrinogenase precursor (VLBF) too (**Figure 19**).

Therefore, full-length cDNAs of RV SBF were constructed from RT-PCR amplification with degenerated primer and 5'RACE with specific primers. The RV SBF nucleotide sequence comprises 1582 basepairs and encodes an open reading frame of 258 amino acids. The translation initiation site was assigned to the first methionine codon ATG (nucleotides 175-177), and the termination codon TGA was found at the nucleotides 949-951. The cDNA includes a 5'-untranslated region (UTR) (174 bp), a signal peptide (54 bp, 18 amino acids), an activation peptide (18 bp, 6 amino acids), a mature enzyme coding region of 702 bp (234 amino acids) and a 3'-UTR (631 bp). The greatest homology occurs with *Macrovipera lebetina* serine beta-fibrinogenase precursor (11) – identity of 91% from the beginning to the 3'-UTR. The identity in the 3'-UTR reached 92% (**Figure 18 and Figure 19**).

Figure 16 An ethidium bromide stained agarose gel showing the PCR product from 5' RACE. Lane M; 300 ng of 100 bp DNA ladder, lane 1; approximately 600 bp of PCR product from 5' RACE.

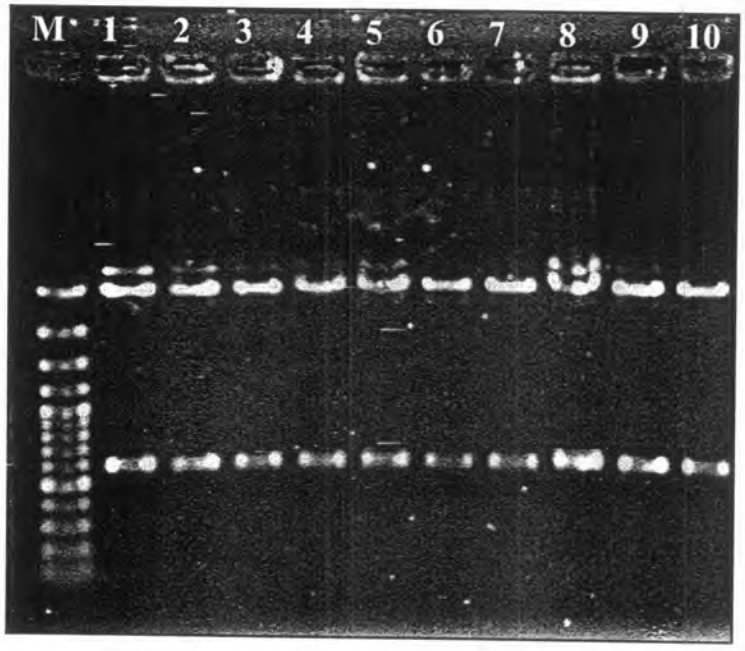


Figure 17 An ethidium bromide stained agarose gel showing *EcoR* I digested recombinant 5' RACE PCR product/pGEM®-T easy vector. Lane M; 100 bp DNA ladder, lane 1-10; approximately 600 bp of 5' RACE PCR product in pGEM®-T easy vector.

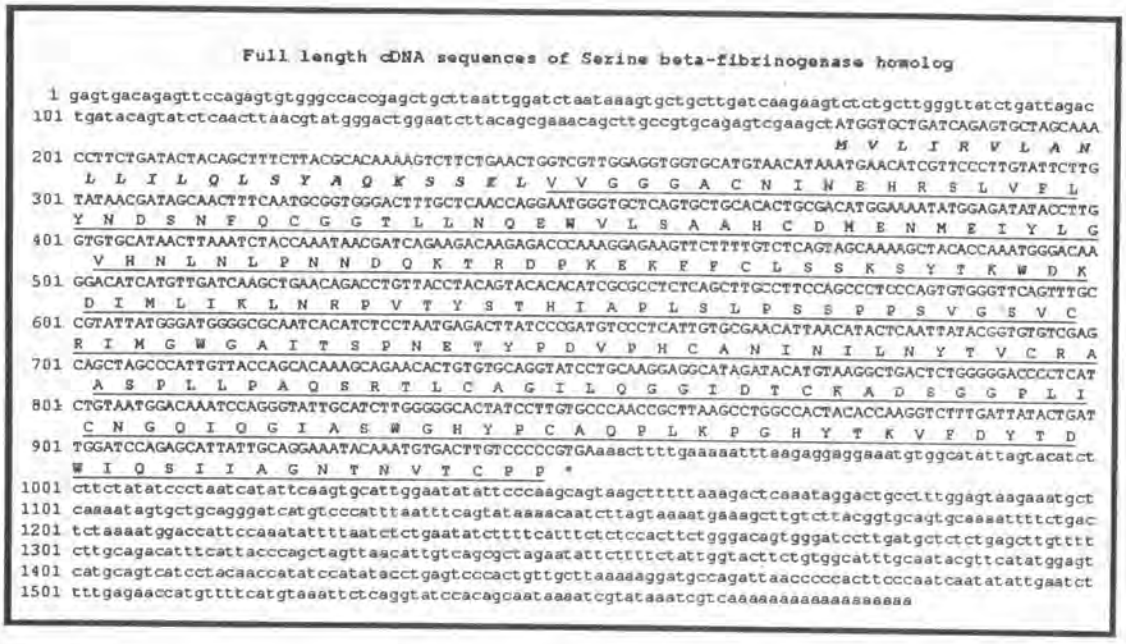


Figure 18 Full length cDNA sequences of RV SBF. Identical partial cDNA sequences. Stop codon was marked with an asterisk (*). The underlined is mature peptide. Signal peptide was shown in Bold and Italic. 5'UTR and 3'UTR sequence were shown in small cabs.

3. Sequences alignment and Computational Analysis of Serine beta-fibrinogenase.

3.1 The cDNA sequence alignment and Phylogenetic tree construct

The cDNA encoding mature peptide sequences were analyzed and comparison to the entries in database using the BLASTN (**Figure 19**) and CLUSTALX program. Multiple alignments between serine beta-fibrinogenase homolog with other 10 highly homology nucleotide sequences, those are highly conserve regions that were shown in black shade boxes in **Figure 20.** The rate of identity with other snake venom serine esterases covers the range of 86-91%.

In addition to Phylogenetic study on sequences similarity between RV SBF and other venom serine protease sequence was assessed using BLASTN .The nucleotide sequences were randomly picked from the GenBank data base. Morover the sequences relationships between 40 serine proteases on the basis of their cDNA encodind mature amino acid sequence from *Viperinae* and *Crotalinae* family. The multiple sequences were aligned by CLUSTALX and a phylogram was constructed by neighbour-joining method with the MEGA 3.1 program. The nodes with bootstrap values of more than 0.70 on the tree are supported with more than 95% probability (**Figure 21**) (Hillis and Bull, 1993).

In this tree reveals major four clusters of serine proteases were separated to six grouds. Majority functions of tree are preferred to thrombin-like enzyme (CL: coagulating enzyme), KN (kininogenase), beta- fibrinogenase and PA (plasminogen activator) and other in subcluster are serine protease that act on blood coagulation cascade.

Additionally, in the first cluster from the left are reveal in group I and group II. Group I are prefer function of KN: kininogenase are containing *Trimeresurus stejnegeri* serine protease KN2 precursor (AF395767), *Trimeresurus stejnegeri* serine protease KN11 precursor (AF395770), *Trimeresurus stejnegeri* serine protease KN3

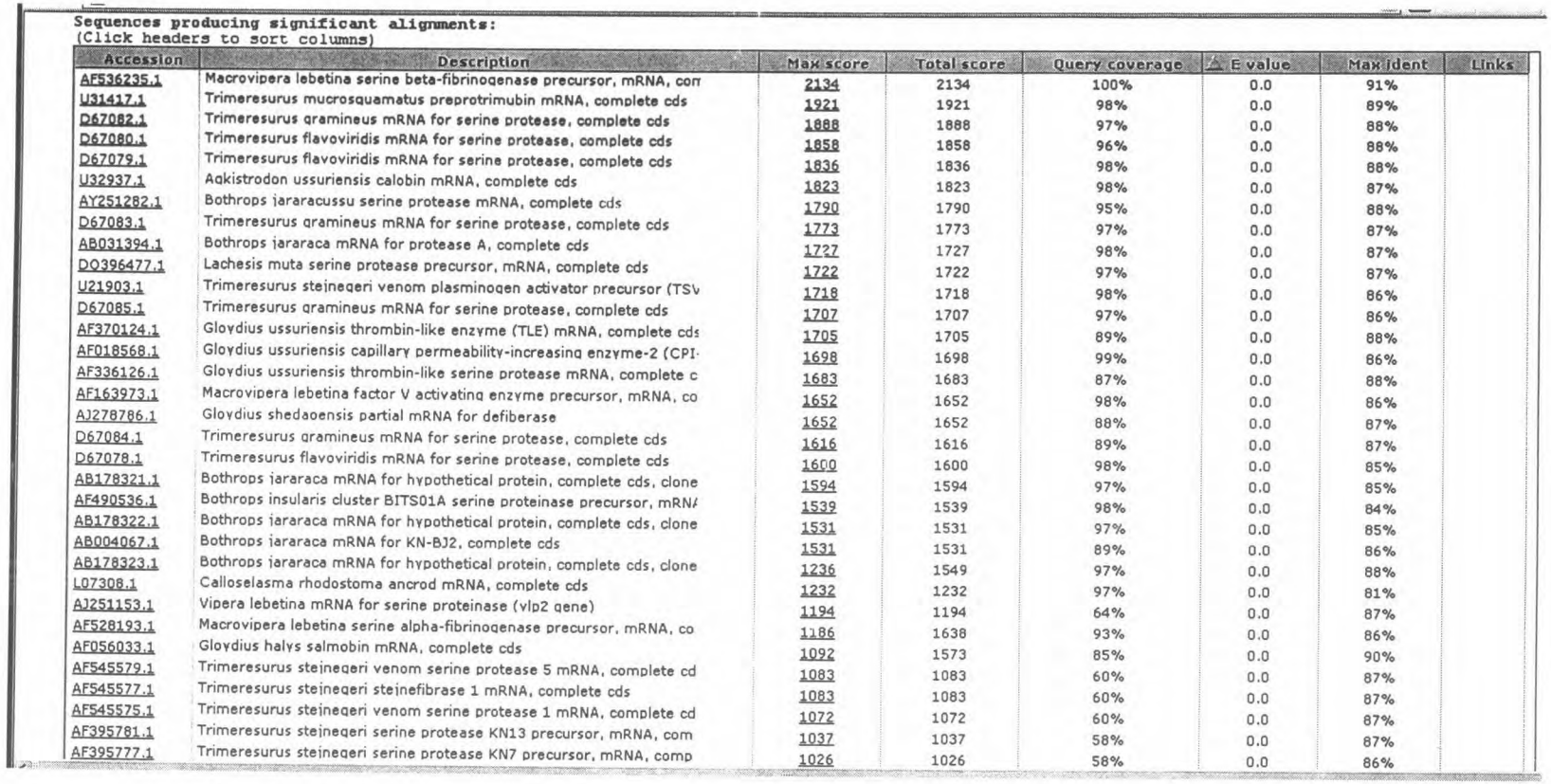
precursor (AF395772), *Trimeresurus stejnegeri* serine protease KN1 precursor (AF395764), *Trimeresurus stejnegeri* serine protease KN8 precursor (AF395782), *Trimeresurus stejnegeri* serine protease KN10 precursor (AF395769)and *Trimeresurus stejnegeri* serine protease KN9 precursor (AF395766). Group II are prefer function of beta-fibrinogenase, those are containing *T.mucrosquamatus* mRNA for mucrofibrase-3 (X83223), *T.mucrosquamatus* mRNA for mucrofibrase-1 (X83221), *T.mucrosquamatus* mRNA for mucrofibrase-2 (X83222), *T.mucrosquamatus* mRNA for mucrofibrase-4 (X83224) and *T.mucrosquamatus* mRNA for mucrofibrase-5 (X83225).

The second clusters or Group III are prefer function of Thrombin-like enzyme that are containing *Gloydius ussuriensis* thrombin-like enzyme (TLE) (AF370124), *Gloydius ussuriensis* thrombin-like serine protease (AF336126), *Gloydius shedaoensis* partial mRNA for defiberase (AJ278786), *Gloydius ussuriensis* thrombin-like enzyme ussurin (AF444250), *Bothrops jararacussu* serine protease (AY251282), *Trimeresurus gramineus* mRNA for serine protease (D67082), *Trimeresurus stejnegeri* venom serine protease 1 (AF545575), *Trimeresurus flavoviridis* mRNA for serine protease (D67079) and *Trimeresurus jerdonii* serine proteinase 1 precursor (AF292110).

The third clusters are reveal in Group IV and Group V. Group IV are prefer function of PA (plasminogen activator) are containing *Trimeresurus jerdonii* serine proteinase 2 precursor (AF292111), *Trimeresurus stejnegeri* venom plasminogen activator precursor (TSV-PA) (U21903), *Trimeresurus jerdonii* serine proteinase 3 precursor (AF292112) and *Lachesis muta* serine protease precursor (DQ396477). Group V are prefer one more function of Thrombin-like enzyme are containing *Gloydius ussuriensis* thrombin-like enzyme defibrase (AY204242), *Gloydius saxatilis* thrombin-like enzyme defibrase (AY204243), *Trimeresurus gramineus* mRNA for serine protease (D67081), *Trimeresurus stejnegeri* serine protease PA precursor (AF395780), *Trimeresurus stejnegeri* stejnefibrase 2

(AF545578), *Deinagkistrodon acutus* venom thrombin-like enzyme (tlet7) (EF101917), *Deinagkistrodon acutus* venom thrombin-like enzyme (tlet3) (EF093561) and *Deinagkistrodon acutus* thrombin-like protein DAV-PA precursor (AF159058)

The last cluster of tree or Group VI are reveal in two sub clusters those are prefer function of beta-fibrinogenase. The first sub cluster are containing *Macrovipera lebetina* serine beta-fibrinogenase precursor (AF536235), *Macrovipera lebetina* serine alpha-fibrinogenase precursor (AF528193) and SBF homolog. And the second sub cluster are containing *Trimeresurus gramineus* mRNA for serine protease (D67085), *Trimeresurus stejnegeri* serine protease CL2 precursor (AF395775), *Trimeresurus flavoviridis* mRNA for serine protease (D67080), *Gloydius halys* thrombin-like enzyme precursor PTLE1 (AY225505) and *Gloydius ussuriensis* capillary permeability-increasing enzyme-2 (CPI-2) (AF018568). Indicate that Serine beta-fibrinogenase homolog will be classification in these groups. Therefore serine beta-fibrinogenase homolog (RV SBF) might be similar on function between serine beta-fibrinogease and serine alpha-fibrinogenase too.



Sequences producing significant alignments:
(Click headers to sort columns)

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
AF536235.1	Macrovipera lebetina serine beta-fibrinogenase precursor, mRNA, com	2134	2134	100%	0.0	91%	
U31417.1	Trimeresurus mucrosquamatus preprotrimubin mRNA, complete cds	1921	1921	98%	0.0	89%	
D67082.1	Trimeresurus gramineus mRNA for serine protease, complete cds	1888	1888	97%	0.0	88%	
D67080.1	Trimeresurus flavoviridis mRNA for serine protease, complete cds	1858	1858	96%	0.0	88%	
D67079.1	Trimeresurus flavoviridis mRNA for serine protease, complete cds	1836	1836	98%	0.0	88%	
U32937.1	Agkistrodon ussuriensis calobin mRNA, complete cds	1823	1823	98%	0.0	87%	
AY251282.1	Bothrops jararacussu serine protease mRNA, complete cds	1790	1790	95%	0.0	88%	
D67083.1	Trimeresurus gramineus mRNA for serine protease, complete cds	1773	1773	97%	0.0	87%	
AB031394.1	Bothrops jararaca mRNA for protease A, complete cds	1727	1727	98%	0.0	87%	
DQ396477.1	Lachesis muta serine protease precursor, mRNA, complete cds	1722	1722	97%	0.0	87%	
U21903.1	Trimeresurus steinegeri venom plasminogen activator precursor (TSV	1718	1718	98%	0.0	86%	
D67085.1	Trimeresurus gramineus mRNA for serine protease, complete cds	1707	1707	97%	0.0	86%	
AF370124.1	Gloydius ussuriensis thrombin-like enzyme (TLE) mRNA, complete cds	1705	1705	89%	0.0	88%	
AF018568.1	Gloydius ussuriensis capillary permeability-increasing enzyme-2 (CPI·	1698	1698	99%	0.0	86%	
AF336126.1	Gloydius ussuriensis thrombin-like serine protease mRNA, complete c	1683	1683	87%	0.0	88%	
AF163973.1	Macrovipera lebetina factor V activating enzyme precursor, mRNA, co	1652	1652	98%	0.0	86%	
AJ278786.1	Gloydius shedaoensis partial mRNA for defibrase	1652	1652	88%	0.0	87%	
D67084.1	Trimeresurus gramineus mRNA for serine protease, complete cds	1616	1616	89%	0.0	87%	
D67078.1	Trimeresurus flavoviridis mRNA for serine protease, complete cds	1600	1600	98%	0.0	85%	
AB178321.1	Bothrops jararaca mRNA for hypothetical protein, complete cds, clone	1594	1594	97%	0.0	85%	
AF490536.1	Bothrops insularis cluster BITS01A serine proteinase precursor, mRNA	1539	1539	98%	0.0	84%	
AB178322.1	Bothrops jararaca mRNA for hypothetical protein, complete cds, clone	1531	1531	97%	0.0	85%	
AB004067.1	Bothrops jararaca mRNA for KN-BJ2, complete cds	1531	1531	89%	0.0	86%	
AB178323.1	Bothrops jararaca mRNA for hypothetical protein, complete cds, clone	1236	1549	97%	0.0	88%	
L07308.1	Calloselasma rhodostoma ancrod mRNA, complete cds	1232	1232	97%	0.0	81%	
AJ251153.1	Vipera lebetina mRNA for serine proteinase (vlp2 gene)	1194	1194	64%	0.0	87%	
AF528193.1	Macrovipera lebetina serine alpha-fibrinogenase precursor, mRNA, co	1186	1638	93%	0.0	86%	
AF056033.1	Gloydius halys salmobin mRNA, complete cds	1092	1573	85%	0.0	90%	
AF545579.1	Trimeresurus steinegeri venom serine protease 5 mRNA, complete cd	1083	1083	60%	0.0	87%	
AF545577.1	Trimeresurus steinegeri steinefibrase 1 mRNA, complete cds	1083	1083	60%	0.0	87%	
AF545575.1	Trimeresurus steinegeri venom serine protease 1 mRNA, complete cd	1072	1072	60%	0.0	87%	
AF395781.1	Trimeresurus steinegeri serine protease KN13 precursor, mRNA, com	1037	1037	58%	0.0	87%	
AF395777.1	Trimeresurus steinegeri serine protease KN7 precursor, mRNA, comp	1026	1026	58%	0.0	86%	

Figure 19 Homology searching of full length cDNA sequences of RV SBF using BLAST N program.

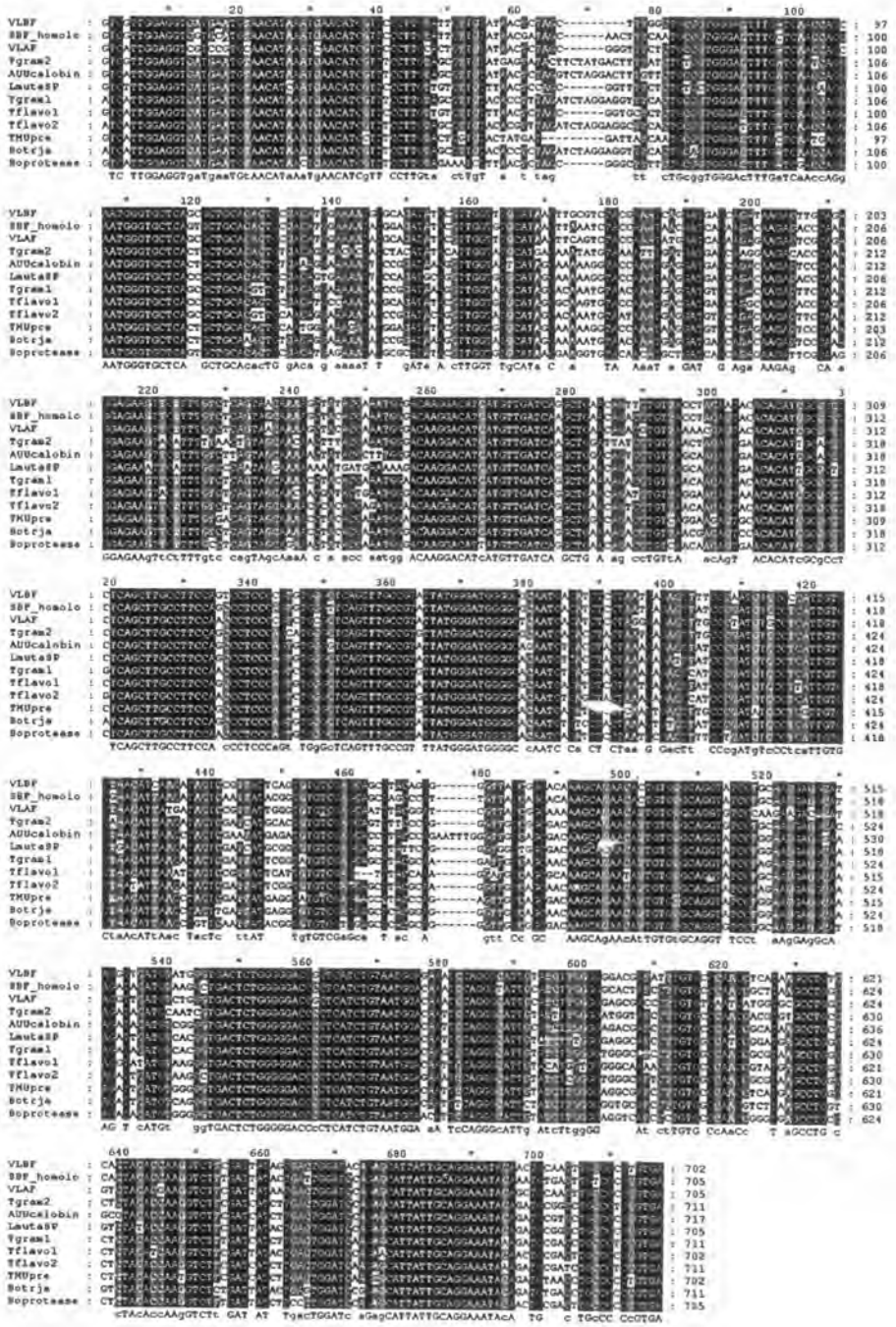


Figure 20 An alignment of cDNA encoding mature peptide between serine RV SBF and other 10 highly homology.

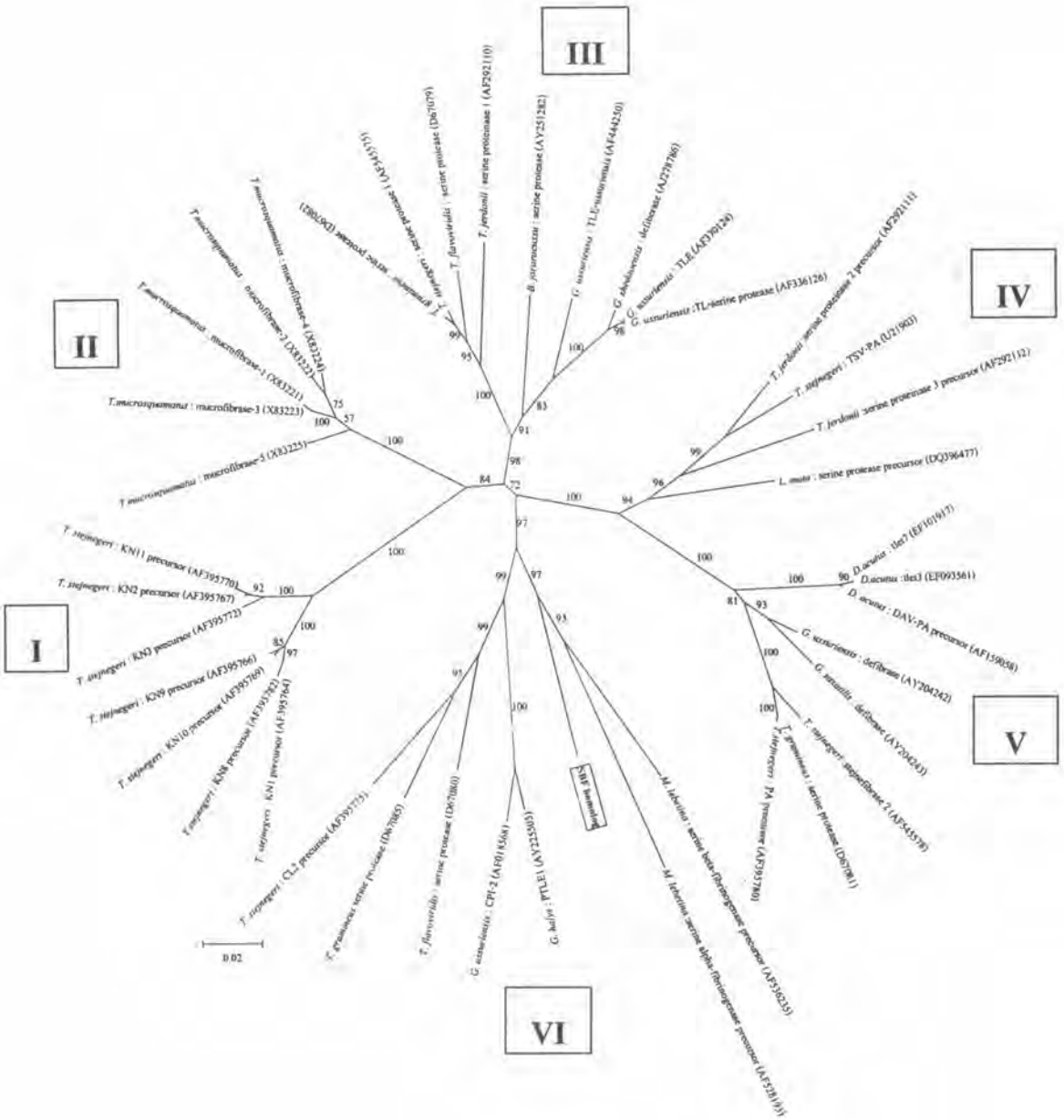


Figure 21 The Phylogenetic tree of snake venom protease. The species name and the GenBank accession number are indicated in the tree.

3.2 Deduced amino acid alignment and computational analysis

By using the BLAST P program, deduced amino acid sequence of Serine beta-fibrinogenase was highest homologous to serine beta-fibrinogenase precursor in *Macrovipera lebetina* venom (AAM96700.1) 80% identical (**Figure 22**).

Deduced amino acid sequence of Serine beta-fibrinogenase homologs were predicted the physical properties using prediction or characterization tools of ExPASy Proteomics tools program via the World Wide Web. Serine beta-fibrinogenase homologs containing 24 amino acids of signal peptide and mature peptide 234 amino acids. The isoelectric point (pI) predictation of deduced amino acid serine beta-fibrinogenase homologs by using Compute pI/Mw Tool (PROSITE program), is 6.68, molecular weight is 25529.1, and amino acid compositions were shown in **Table 3** and **Figure 23**, That have total number of negatively charged residues (Asp + Glu) and positively charged residues (Arg + Lys) 17, 16 respectively. Additional, conserve domain characterization of serine beta-fibrinogenase homologs was similarity to Serine proteases, trypsin domain. That were cleaves preferentially to Arg-|-Xaa, Lys-|-Xaa position of substrate.

Furthermore, serine beta-fibrinogenase homologs have similarity catalytic triad at His-41, Asp-86, Ser-180 and form 4 disulfide bonds [condition: C-x*-C] are Cys-26±Cys-42, Cys-118±Cys-186, Cys-150±Cys-165, Cys-176±Cys-201. And The predict N-glycosylation () was contained 4 position, Asn-44 were formed NDSN, Asn-154 were formed NETY, Asn-170 were formed NYTV and Asn-253 were formed NVTC by similarity. Those were shown in **Figure 24**. In addition, the alignment mature peptide sequence of Serine beta-fibrinogenase homolog with other 10 highly homology. Those were shown catalytic triad and 12 cystein residues highly conserve in serine protease group and were showed highly conserve regions in black shade boxes of **Figure 25**.

Sequences producing significant alignments:	Score (Bits)	E Value
gb|AAM96700.1| serine beta-fibrinogenase precursor [Macrovipera	392	1e-107
gb|AAL68708.1|AF336126_1 thrombin-like serine protease [Gloyd...	374	2e-102
sp|Q9PTU8|VSP3_BOTJA Venom serine proteinase A precursor >dbj...	371	1e-101
gb|AAN52348.1| stejnefibrase 1 [Trimeresurus stejnegeri]	371	2e-101
gb|AAQ02906.1| serine protease KN12 precursor [Trimeresurus stej	369	5e-101
gb|AAQ02904.1| serine protease CL5 precursor [Trimeresurus stejn	369	6e-101
emb|CAB65936.1| beta-fibrinogenase [Gloydius blomhoffi]	367	2e-100
gb|AAQ02909.1| serine protease CL4 precursor [Trimeresurus stejn	367	3e-100
gb|AAQ02911.1| serine protease KN13 precursor [Trimeresurus stej	366	6e-100
gb|AAN52350.1| venom serine protease 5 [Trimeresurus stejnegeri]	365	9e-100
gb|AAF25008.1|AF176679_1 salmonase [Gloydius halys brevicaudus]	365	1e-99
gb|AAQ02893.1| serine protease KN4 precursor [Trimeresurus stejn	363	3e-99
sp|O13060|VSPA_TRIGA Venom serine proteinase 2A precursor >db...	363	4e-99
sp|O42207|VSP2_AGKCA Capillary permeability-increasing enzyme...	363	6e-99
gb|AAL48221.1| thrombin-like enzyme ussurin [Gloydius ussuriensi	362	9e-99
gb|AAQ02907.1| serine protease KN7 precursor [Trimeresurus stejn	362	1e-98
sp|O13063|VSP3_TRIGA Venom serine proteinase 3 precursor >dbj...	360	2e-98
gb|AAL48222.1| thrombin-like enzyme ussurase [Gloydius ussuriens	360	3e-98
gb|AAM96674.1| serine alpha-fibrinogenase precursor [Macrovipera	360	3e-98

Figure 22 Homology searching of full length cDNA sequences of serine beta-fibrinogenase homolog using BLAST N program.

Table 5 The Amino acid composition of serine beta-fibrinogenase homolog

Amino acid	Number of amino acids	%	Amino acid	Number of amino acids	%
Ala (A)	14	6.0%	Gln (Q)	9	3.8%
Arg (R)	6	2.6%	Glu (E)	6	2.6%
Asn (N)	18	7.7%	Gly (G)	19	8.1%
Asp (D)	11	4.7%	His (H)	7	3.0%
Cys (C)	12	5.1%	Ile (I)	17	7.3%
Leu (L)	20	8.5%	Ser (S)	18	7.7%
Lys (K)	10	4.3%	Thr (T)	14	6.0%
Met (M)	4	1.7%	Trp (W)	5	2.1%
Phe (F)	5	2.1%	Tyr (Y)	9	3.8%
Pro (P)	18	7.7%	Val (V)	12	5.1%

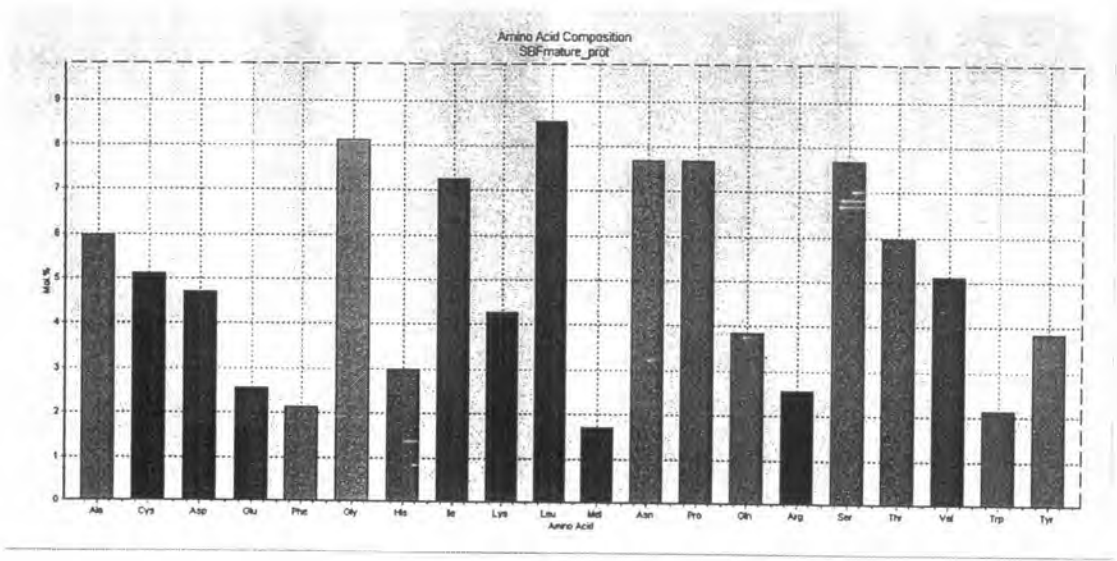


Figure 23 The Amino acid composition of serine beta-fibrinogenase homolog

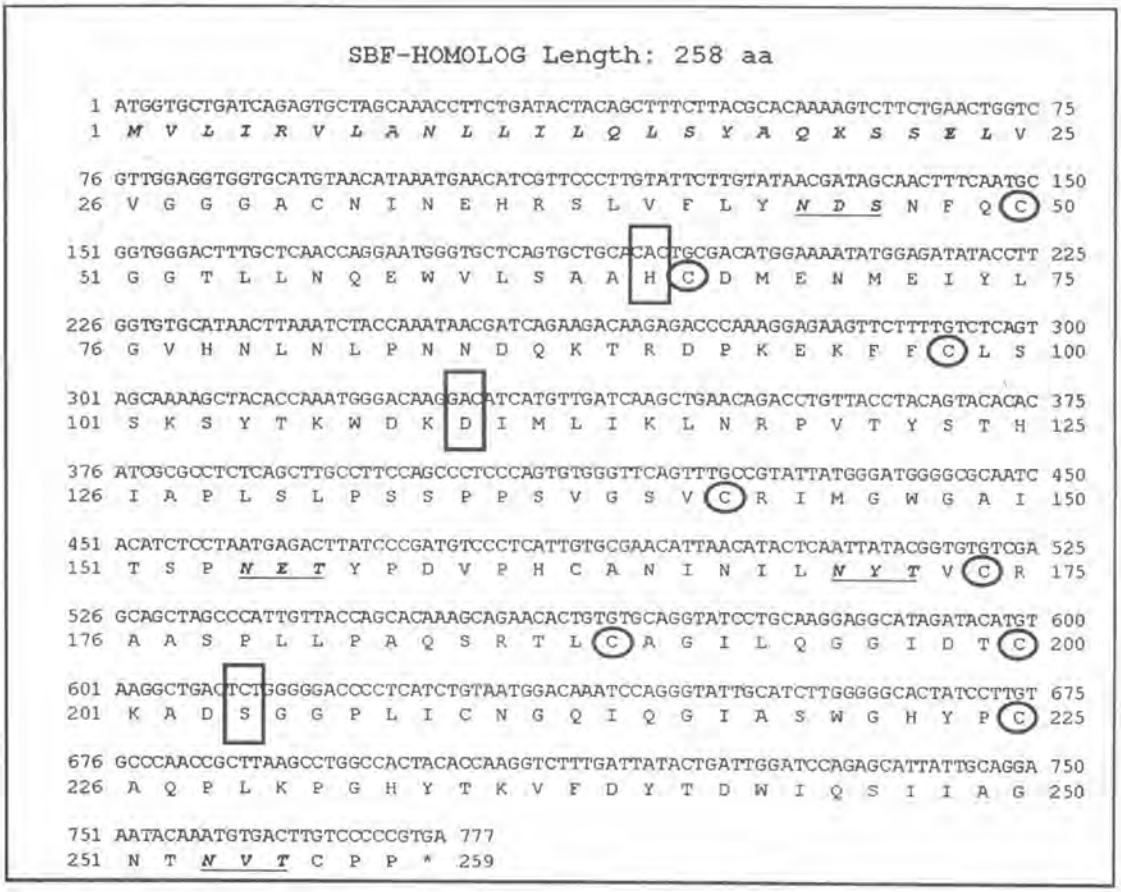


Figure 24 Predicted physical properties of deduced amino acid Serine beta-fibrinogenase homolog. Predicted N-glycosylation positions are underlined. The residues of conserve catalytic triad are boxed. The cysteine residues for form disulfide bond are indicated by open circles.

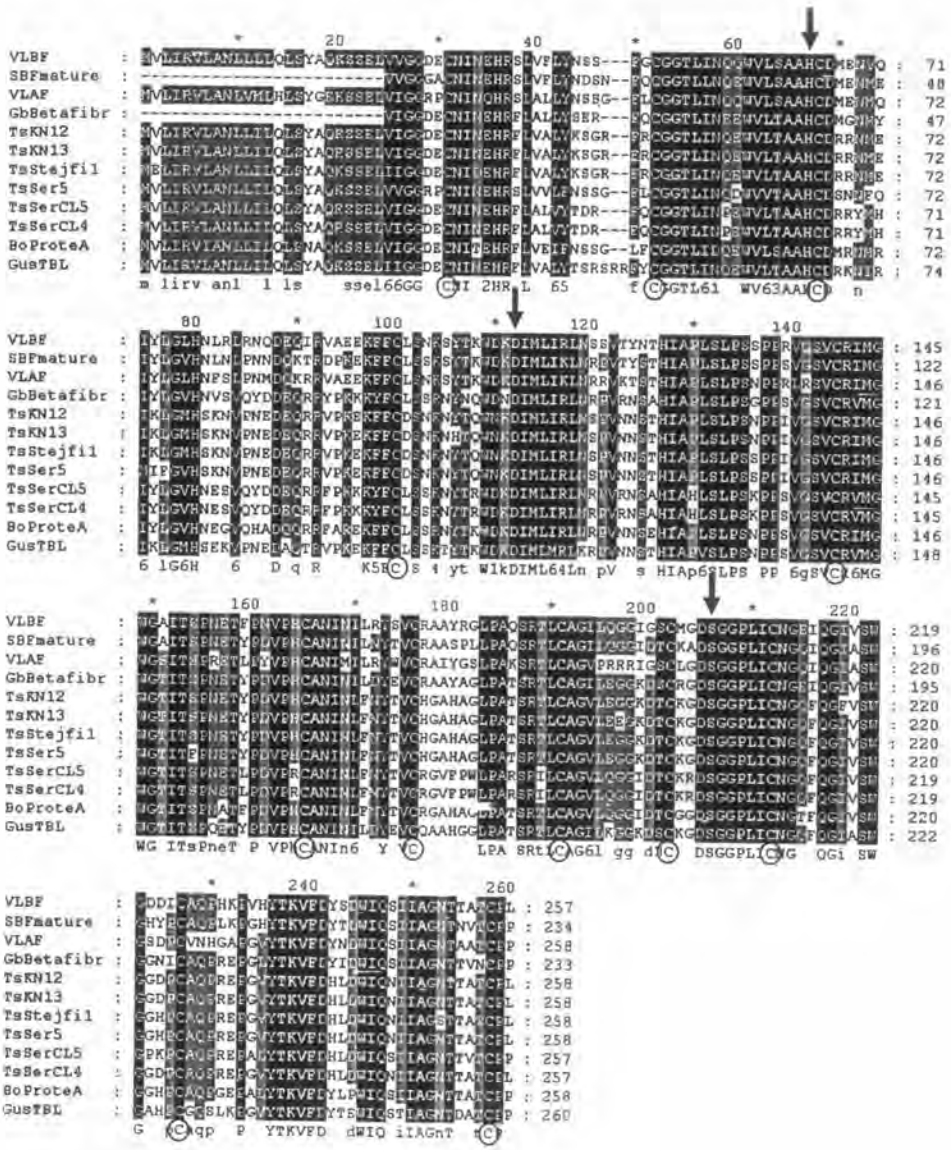


Figure 25 The active protein sequences alignment between Serine beta-fibrinogenase homolog and other 10 highly similarity sequences.