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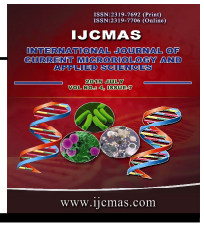

Original Research Article

Characterization of a Gene Encoding Serrapeptidase from *Serratia marcescens* Strain (SRM) MTCC 8708, a Plant Isolate

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**Corresponding author*

K e y w o r d s

Serrapeptidase, *Serratia marcescens*, Gene sequence, Metalloprotease, Microbial protease, *Enterobacterium*

A B S T R A C T

Serrapeptidase is pharmaceutically, entomologically important protease isolated from *Serratia marcescens*, a saprophytic organism. Presently available pharmaceutical product, used as an anti-inflammatory drug, has been isolated from *Serratia piscatorum*, an entrobacterium found in intestinal canal of silk worm. Here we report the characterization of the gene isolated from an organism of plant source with an objective to understand the biochemical basis of its encoded protein. The genomic DNA of *Serratia marcescens* MTCC 8708 was isolated using standard protocol and used as a source for the gene. Primers corresponding to Serrapeptidase were designed based on the sequences available in the data base and gene amplified. A single amplified DNA of 1.5Kb was purified and cloned into pJET 1.2 cloning vector and subjected for sequencing. The sequence analysis revealed the presence of single Open reading frame (ORF) of 1464 nucleotide with high G+C content (58%) encoding a protein of 487 amino acid residues. The sequence alignment using the BLAST search in NCBI Gen Bank showed 100% homology with serralysin metalloprotease from *Serratia marcescens* strain 2170. The derived amino acid sequence was further analyzed for conserved domains and motifs in database which showed the presence of three conserved domains for Zinc binding site and Histidine amino acid at active site. The similarities of these regions with other proteases like thermolysin, and a neutral protease of *Bacillus subtilis*, suggest that the derived amino acid sequence from the gene may correspond to a metalloprotease. The gene may be used to produce a protein for pesticidal / pharmaceutical application.

Introduction

Serrapeptidase is a metalloprotease (EC.3.4.24.40), first isolated from *Serratia piscatorum*, an entrobacterium found in intestinal canal of silk worm (Kodama *et al.*, 1965). The enzyme was tentatively named as Serratia peptidase for the reason of origin (Miyata *et al.*, 1971). Subsequently this enzyme was also isolated from *Serratia marcescens* ATCC 25419 (Decedue *et al.*, 1979). *Serratia marcescens* is a gram

negative entrobacteria characterized by its pink / red colony (Grimont and Grimont, 1984). It is omnipotent, found in water, soil, bathtub, and sink etc. (Bayona *et al.*, 2008–2009). A few species of entrobacteria secrets protease extracellular, one such species is *Serratia marcescans* (Schmitz and Braun, 1985).

According to Matsumoto *et al.* (1984) clinical strains of *Serratia* species produce more than four proteases. The major metalloprotease (51 Kda) has anti inflammatory activity. This enzyme breaks down the protein debris which includes toxins and other molecules released in inflammation (Yamasaki *et al.*, 1967). It acts especially on dead cells such as scar tissue, blood clot, cyst, mucus (Nakamura *et al.*, 2003), arterial plaque and inflammated cells. Dr. Hans Nieper identified the wonderful property of serrapeptidase and subsequently named it as 'Miracle Enzyme', which can be used to dissolve blood clots and shrink/ diminish varicose veins (Alexander III and and Eagle-Oden, 1999). After having identified the potentiality of serrapeptidases in pharmaceutical applications, attempts have been made to isolate and purify the major protease for subsequent use not only as therapeutic agent but also in insecticidal application (Mohankumar and Hari Krishna Raj, 2011; Tao *et al.*, 2007; Nakahama *et al.*, 1986). However the conventional methods of isolating from the parent organism have several limitations like, high cost involvement, due to low amount present in the culture. Also possible presence of impurities may cause problems in application. More over purification to homogeneity is not possible by conventional methods of isolation from the cultured organism, and homogenous preparation is needed for above applications and also for conducting further studies on its characterization. Thus the well known rDNA approach is ideal, need based and will have no problem for getting regulatory approval for subsequent application.

As a preliminary step in present work, the gene of serratiopeptidase was amplified from genome of *Serratia marcescens* MTCC 8708 (Selvakumar *et al.*, 2008) and cloned and sequenced. The sequence was further analyzed by bioinformatics tools to understand its characteristic features.

Materials and Methods

Isolation of genomic DNA

Genomic DNA used in this study was isolated from *Serratia marcescens* strain SRM MTCC 8708 isolated from flowers of summer squash (*cucubita pepa*). Genomic DNA was isolated by using Tri-Xtract (G-Biosciences St. Leuis, Mo, USA) as per manufacturer's protocol. Briefly, single colony of the bacteria was inoculated in LB broth and grown over night. About $1X10^7$ (2 mL) bacterial cells were collected by centrifugation, 1 mL of Tri-extract was added and the cells were lysed by repeated pipetting. Following incubation at room temperature for 5 min, 200 μL of chloroform was added and the contents were mixed vigorously for 15 seconds. The mixture was incubated for 30 minutes, centrifuged at 12, 000 g for 15 min at 4^oC.

The centrifuged tube contains a lower pink, a colorless upper aqueous phase and phenol-chloroform at interphase. The aqueous phase containing RNA was discarded. The organic phase containing DNA and protein as impurities was purified by adding 300 μL of 100% ethanol and incubated at room temperature for 3 min. The mixture was centrifuged at 2000g for 5 min at 4^oC. The phenol/ethanol supernatant was carefully removed and the pelleted DNA was washed

with 1 mL 0.1M sodium citrate in 10% ethanol at room temperature. The mixture was centrifuged again at 2000g for 5 min. For the final wash, the pellet was suspended in 1.5 mL 70% ethanol and then centrifuged at 2000g for 5 min at 4^{o}C and the DNA pellet was air dried for 15 min. Finally, the DNA was resuspended in 300μL of 8mM NaOH to give a final concentration of 0.3μg/μL. The final pH was adjusted to 8.4 with HEPES buffer for PCR amplification.

Amplification of serrapeptidase gene by PCR

The serrapeptidase gene was amplified using specific forward [Ser pep (L): 5' GGAAGCTTCATATGCAATCTACTAAA AAGGCAAT] and reverse [Ser pep (R): 5'GCGAAGCTTACACGATAAAGTAGT GGCGACGT] primers which were designed based on the sequence available in database. The primers were used at working dilution of 20pm/μl in sterile filtered water (FQW).

Optimum annealing temperature was determined by employing gradient PCR. Amplification reaction was carried out in 0.5 ml thin walled PCR tubes in a reaction mixture of 50 μl which consisted of 20 pm of each primer, 25 mM Tris - HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200mM each of four dNTPS, 100ng template DNA and 1 unit of Taq DNA polymerase (Accu. Taq, Sigma). The PCR conditions used were; initial denaturation at 95^{o}C for 5 min, followed by 35 thermal cycles, each with denaturation at 94^{o}C for 1 min, annealing at 60^{o}C for 1 min and extension at 72^{o}C for 3 min with a final extension at 72^{o}C for 10 min in an Eppendorf Master Cycler 5330 (Germany). No template DNA was used in negative control. The amplified DNA fragments were runned in 1% agarose gel electrophoresis along with negative control.

Cloning of serrapeptidase gene in pJET 1.2 cloning vector

PCR product was purified from Agarose gel using GeneJET PCR Purification Kit (# k0701, Thermo Scientific, USA). The overhangs generated by Taq DNA polymerase in purified PCR fragment was removed using DNA blunting enzyme. The blunting reaction mixture contained about 1 μl purified DNA, 10 μL of 2X reaction buffer, 17 μL of nuclease free water and 1 μL DNA blunting enzyme (Thermo Scientific, USA). The contents were mixed by vortexing, centrifuged for 3-5 seconds and incubated at 70^{o}C for 5min. The ligation reaction was carried out by adding 1 μl of pJET 1.2 cloning vector and 1 μL T4 DNA ligase enzyme with the blunted PCR product. The ligation mixture was kept at 22^{o}C for 5 min. The ligated mixture (1 μl) was transferred into competent *E. coli* DH α cells and plated on LB agar containing 50μg/mL ampicillin. The plates were kept overnight in an incubator at 37^{o}C.

Sequencing and sequence analysis

Colonies were screened by colony lysis and colony PCR. Plasmid DNAs were prepared from two of the positive clones and were subjected for sequencing at the facility available with Chromous Biotech Pvt Ltd., Bangalore, India, using ABI 3730 XL Genetic Analyzer. Sequence analysis was performed using the NCBI online programme "BLAST" (http://blast.ncbi. nlm.nih.gov/).

Results and Discussion

Amplification of 1.5 Kb serrapeptidase gene by PCR:

Genomic DNA of *Serratia marcescens*

(MTCC 8707) was extracted as described above. Serrapeptidase gene was amplified using suitable primers. The amplified DNA of 1.5 kb was analyzed by 1% Agarose gel electrophoresis ((Fig. 1). As seen in the figure, 2nd lane, a single intense band of size 1.5 kb was visible in the case of positive reaction with DNA template which is not seen negative control, indicating that the PCR product is the specific gene amplified from the template using serrapeptidase specific primers.

Cloning of serrapeptidase gene in pJET 1.2 cloning vector

The purified PCR product was ligated into pJET1.2 cloning vector. The ligated mixture was transferred into competent *E. coli* DH 5α and plated on LB agar plate (Ampillicin resistance). Only cells with recombinant plasmids were propagated because pJET 1.2 vector contains a lethal gene, which disrupted by ligation of a DNA inserted into cloning site. Above 10 transformants with white colonies were observed. The white colonies were subjected to lysis and analyzed by 1% Agarose gel stained with ethidium bromide to check there mobility. Plasmids from all the 10 were found to be migrating slower than the control vector (Figure not shown), indicating that these are positive for the presence of inserted DNA. Plasmid DNAs from two of the positive colonies were purified and further studied for the presence of serrapeptidase gene by sequence analysis.

Fig. 1 Agarose gel electophoresis of amplified gene

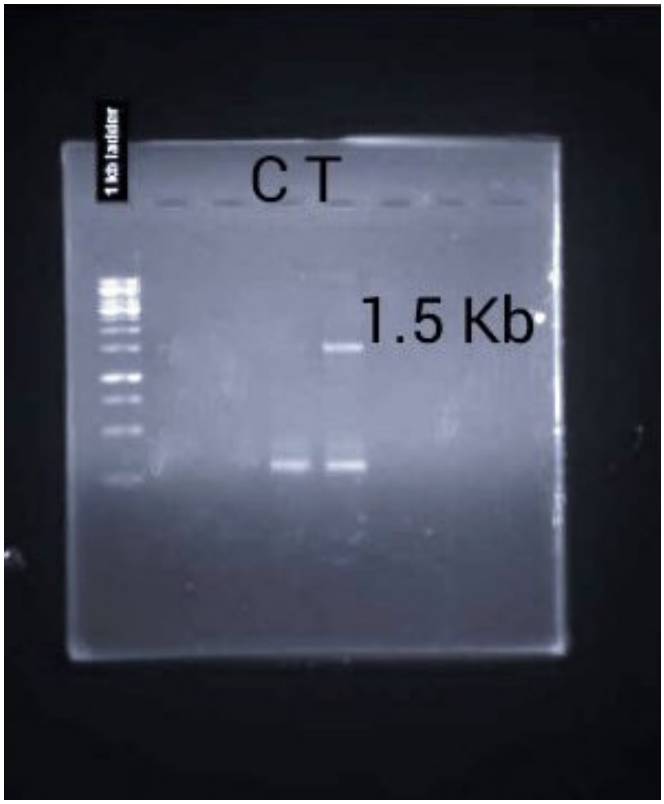

Fig. 2 Nucleotide sequence of the cloned serratio peptidase gene [initiation codon (ATG) and termination site (TAA) underlined]

```
ORIGIN
       1 ATGCAATCTA CTAAAAAGGC AATTGAAATT ACTGAATCCA GCCTCGCTGC CGCGACAACC
      61 GGTTACGATG CTGTAGACGA CCTGCTGCAT TATCATGAGC GGGGTAACGG GATTCAGATT
     121 AATGGCAAGG ATTCATTTTC TAACGAGCAA GCTGGGCTGT TTATTACCCG TGAGAACCAA
     181 ACCTGGAACG GTTACAAGGT ATTTGGCCAG CCGGTCAAAT TAACCTTCTC GTTCCCGGAC
     241 TATAAGTTCT CTTCCACCAA CGTCGCCGGC GACACCGGGC TGAGCAAGTT CAGCGCGGAA
     301 CAGCAGCAGC AGGCTAAGCT GTCGCTGCAG TCCTGGGCCG ACGTCGCCAA TATCACCTTC
     361 ACCGAAGTGG CGGCCGGTCA AAAGGCCAAT ATCACCTTCG GCAATTACAG CCAGGATCGT
     421 CCCGGCCACT ATGATTACGG CACCCAGGCC TACGCCTTCC TGCCGAACAC CATTTGGCAG
     481 GGCCAGGATT TGGGCGGCCA GACCTGGTAC AACGTCAACC AATCCAACGT GAAGCATCCG
     541 GCGACCGAAG ACTACGGCCG CCAGACGTTC ACCCATGAGA TTGGCCATGC GCTGGGCCTG
     601 AGCCACCCGG GCGACTACAA CGCCGGTGAG GGCAACCCGA CCTATAGAGA TGTCACCTAT
     661 GCGGAAGATA CCCGCCAGTT CAGCCTGATG AGCTACTGGA GTGAAACCAA TACCGGTGGC
     721 GACAACGGCG GTCACTATGC CGCGGCTCCG CTGCTGGATG ACATTGCCGC CATTCAGCAT
     781 CTGTATGGCG CCAACCTGTC GACCCGCACC GGCGACACCG TGTACGGCTT TAACTCCAAT
     841 ACCGGTCGTG ACTTCCTCAG CACCACCAGC AACTCGCAGA AAGTGATCTT TGCGGCCTGG
     901 GATGCGGGCG GCAACGATAC CTTCGACTTC TCCGGTTACA CCGCTAACCA GCGCATCAAC
     961 CTGAACGAGA AATCGTTCTC CGACGTGGGC GGCCTGAAGG GCAACGTCTC GATCGCCGCC
    1021 GGTGTGACCA TTGAGAACGC CATTGGCGGT TCCGGCAACG ACGTGATCGT CGGCAACGCG
    1081 GCCAACAACG TGCTGAAAGG CGGCGCGGGT AACGACGTGC TGTTCGGCGG CGGCGGGGCG
    1141 GATGAATTGT GGGGCGGTGC CGGCAAAGAC ATCTTCGTGT TCTCTGCCGC CAGCGATTCC
    1201 GCACCGGGCG CTTCAGACTG GATCCGCGAC TTCCAGAAGG GGATCGACAA GATCGACCTG
    1261 TCGTTCTTCA ATAAAGAAGC GCAGAGCAGC GATTTCATTC ACTTCGTCGA TCACTTCAGC
    1321 GGCACGGCCG GTGAGGCGCT GCTGAGCTAC AACGCGTCCA GCAACGTGAC AGATTTGTCG
    1381 GTGAACATCG GTGGGCATCA GGCGCCGGAC TTCCTGGTGA AAATCGTCGG CCAGGTAGAC
    1441 GTCGCCACTG ACTTTATCGT GTAA
```

Fig.3 Sequence alignment showing homology between the cloned Serrapeptidase gene and corresponding gene of *Serratia marcescens strain: 2170*

Select seq dbj|AB873002.1 *Serratia marcescens* ser gene for serralysin metalloprotease, complete cds, strain: 2170

Length: 1482
Features in this part of subject sequence:
Serratio peptidase

Score = 2687 bits(1455), Expect = 0.0
Identities = 1461/1464(99%), Gaps = 0/1464(0%)
Strand = Plus / Plus

```
Query  1     ATGCAATCTACTAAAAAGGCAATTGAAATTACTGAATCCAGCCTCGCTGCCGCGACAACC  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  19    ATGCAATCTACTAAAAAGGCAATTGAAATTACTGAATCCAGCCTCGCTGCCGCGACAACC  78

Query  61    GGTTACGATGCTGTAGACGACCTGCTGCATTATCATGAGCGGGGTAACGGGATTCAGATT 120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  79    GGTTACGATGCTGTAGACGACCTGCTGCATTATCATGAGCGGGGTAACGGGATTCAGATT 138

Query  121   AATGGCAAGGATTCATTTTCTAACGAGCAAGCTGGGCTGTTTATTACCCGTGAGAACCAA 180
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Sbjct  139   AATGGCAAGGATTCATTTTCTAACGAGCAAGCTGGGCTGTTTATTACCCGTGAGAACCAA 198
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Query  181   ACCTGGAACGGTTACAAGGTATTTGGCCAGCCGGTCAAATTAACCTTCTCGTTCCCGGAC 240
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Query  241   TATAAGTTCTCTTCCACCAACGTCGCCGGCGACACCGGGCTGAGCAAGTTCAGCGCGGAA 300
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Sbjct  259   TATAAGTTCTCTTCCACCAACGTTGCCGGCGACACCGGGCTGAGCAAGTTCAGCGCGGAA 318

Query  301   CAGCAGCAGCAGGCTAAGCTGTCGCTGCAGTCCTGGGCCGACGTCGCCAATATCACCTTC 360
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  319   CAGCAGCAGCAGGCTAAGCTGTCGCTGCAGTCCTGGGCCGACGTCGCCAATATCACCTTC 378

Query  361   ACCGAAGTGGCGGCCGGTCAAAAGGCCAATATCACCTTCGGCAATTACAGCCAGGATCGT 420
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  379   ACCGAAGTGGCGGCCGGTCAAAAGGCCAATATCACCTTCGGCAATTACAGCCAGGATCGT 438

Query  421   CCCGGCCACTATGATTACGGCACCCAGGCCTACGCCTTCCTGCCGAACACCATTTGGCAG 480
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Query  481   GGCCAGGATTTGGGCGGCCAGACCTGGTACAACGTCAACCAATCCAACGTGAAGCATCCG 540
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Query  541   GCGACCGAAGACTACGGCCGCCAGACGTTCACCCATGAGATTGGCCATGCGCTGGGCCTG 600
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Sbjct  559   GCGACCGAAGACTACGGCCGCCAGACGTTCACCCATGAGATTGGCCATGCGCTGGGCCTG 618

Query  601   AGCCACCCGGGCGACTACAACGCCGGTGAGGGCAACCCGACCTATAGAGATGTCACCTAT 660
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Query  661   GCGGAAGATACCCGCCAGTTCAGCCTGATGAGCTACTGGAGTGAAACCAATACCGGTGGC 720
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Query  721   GACAACGGCGGTCACTATGCCGCGGCTCCGCTGCTGGATGACATTGCCGCCATTCAGCAT 780
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Query  781   CTGTATGGCGCCAACCTGTCGACCCGCACCGGCGACACCGTGTACGGCTTTAACTCCAAT 840
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  799   CTGTATGGCGCCAACCTGTCGACCCGCACCGGCGACACCGTGTACGGCTTTAACTCCAAT 858

Query  841   ACCGGTCGTGACTTCCTCAGCACCACCAGCAACTCGCAGAAAGTGATCTTTGCGGCCTGG 900
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Sbjct  859   ACCGGTCGTGACTTCCTCAGCACCACCAGCAACTCGCAGAAAGTGATCTTTGCGGCCTGG 918

Query  901   GATGCGGGCGGCAACGATACCTTCGACTTCTCCGGTTACACCGCTAACCAGCGCATCAAC 960
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  919   GATGCGGGCGGCAACGATACCTTCGACTTCTCCGGTTACACCGCTAACCAGCGCATCAAC 978

Query  961   CTGAACGAGAAATCGTTCTCCGACGTGGGCGGCCTGAAGGGCAACGTCTCGATCGCCGCC1020
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  979   CTGAACGAGAAATCGTTCTCCGACGTGGGCGGCCTGAAGGGCAACGTCTCGATCGCCGCC1038
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Query  1021  GGTGTGACCATTGAGAACGCCATTGGCGGTTCCGGCAACGACGTGATCGTCGGCAACGCG1080
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Query  1081  GCCAACAACGTGCTGAAAGGCGGCGCGGGTAACGACGTGCTGTTCGGCGGCGGCGGGGCG1140
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Query  1141  GATGAATTGTGGGGCGGTGCCGGCAAAGACATCTTCGTGTTCTCTGCCGCCAGCGATTCC1200
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Sbjct  1159  GATGAATTGTGGGGCGGTGCCGGCAAAGACATCTTCGTGTTCTCTGCCGCCAGCGATTCC1218

Query  1201  GCACCGGGCGCTTCAGACTGGATCCGCGACTTCCAGAAGGGGATCGACAAGATCGACCTG1260
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Query  1261  TCGTTCTTCAATAAAGAAGCGCAGAGCAGCGATTTCATTCACTTCGTCGATCACTTCAGC1320
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1279  TCGTTCTTCAATAAAGAAGCGCAGAGCAGCGATTTCATTCACTTCGTCGATCACTTCAGC1338

Query  1321  GGCACGGCCGGTGAGGCGCTGCTGAGCTACAACGCGTCCAGCAACGTGACAGATTTGTCG1380
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Query  1381  GTGAACATCGGTGGGCATCAGGCGCCGGACTTCCTGGTGAAAATCGTCGGCCAGGTAGAC1440
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Sbjct  1399  GTGAACATCGGTGGGCATCAGGCGCCGGACTTCCTGGTGAAAATCGTCGGCCAGGTAGAC1458

Query  1441  GTCGCCACTGACTTTATCGTGTAA  1464
             |||||||| ||||||||||||||
Sbjct  1459  GTCGCCACGGACTTTATCGTGTAA  1482
```

Fig.4 Deduced amino acid sequence of *Serratia marcescens* serrapeptidase gene (Zinc ligand binding domains are underlined and * active site amino acid)

```
   1 ATGCAATCTACTAAA AAGGCAATTGAAATT ACTGAATCCAGCCTC GCTGCCGCGACAACC
   1  M  Q  S  T  K   K  A  I  E  I   T  E  S  S  L   A  A  A  T  T
  61 GGTTACGATGCTGTA GACGACCTGCTGCAT TATCATGAGCGGGGT AACGGGATTCAGATT
  21  G  Y  D  A  V   D  D  L  L  H   Y  H  E  R  G   N  G  I  Q  I
 121 AATGGCAAGGATTCA TTTTCTAACGAGCAA GCTGGGCTGTTTATT ACCCGTGAGAACCAA
  41  N  G  K  D  S   F  S  N  E  Q   A  G  L  F  I   T  R  E  N  Q
 181 ACCTGGAACGGTTAC AAGGTATTTGGCCAG CCGGTCAAATTAACC TTCTCGTTCCCGGAC
  61  T  W  N  G  Y   K  V  F  G  Q   P  V  K  L  T   F  S  F  P  D
 241 TATAAGTTCTCTTCC ACCAACGTCGCCGGC GACACCGGGCTGAGC AAGTTCAGCGCGGAA
  81  Y  K  F  S  S   T  N  V  A  G   D  T  G  L  S   K  F  S  A  E
 301 CAGCAGCAGCAGGCT AAGCTGTCGCTGCAG TCCTGGGCCGACGTC GCCAATATCACCTTC
 101  Q  Q  Q  Q  A   K  L  S  L  Q   S  W  A  D  V   A  N  I  T  F
 361 ACCGAAGTGGCGGCC GGTCAAAAGGCCAAT ATCACCTTCGGCAAT TACAGCCAGGATCGT
 121  T  E  V  A  A   G  Q  K  A  N   I  T  F  G  N   Y  S  Q  D  R
 421 CCCGGCCACTATGAT TACGGCACCCAGGCC TACGCCTTCCTGCCG AACACCATTTGGCAG
 141  P  G  H  Y  D   Y  G  T  Q  A   Y  A  F  L  P   N  T  I  W  Q
 481 GGCCAGGATTTGGGC GGCCAGACCTGGTAC AACGTCAACCAATCC AACGTGAAGCATCCG
 161  G  Q  D  L  G   G  Q  T  W  Y   N  V  N  Q  S   N  V  K  H  P
 541 GCGACCGAAGACTAC GGCCGCCAGACGTTC ACCCATGAGATTGGC CATGCGCTGGGCCTG
 181  A  T  E  D  Y   G  R  Q  T  F   T *H  E  I  G  *H  A  L  G  L
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```
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 201  S  H  P  G  D   Y  N  A  G  E   G  N  P  T  Y   R  D  V  T  Y
 661 GCGGAAGATACCCGC CAGTTCAGCCTGATG AGCTACTGGAGTGAA ACCAATACCGGTGGC
 221  A  E  D  T  R   Q  F  S  L  M   S  Y  W  S  E   T  N  T  G  G
 721 GACAACGGCGGTCAC TATGCCGCGGCTCCG CTGCTGGATGACATT GCCGCCATTCAGCAT
 241  D  N  G  G  H   Y  A  A  A  P   L  L  D  D  I   A  A  I  Q  H
 781 CTGTATGGCGCCAAC CTGTCGACCCGCACC GGCGACACCGTGTAC GGCTTTAACTCCAAT
 261  L  Y  G  A  N   L  S  T  R  T   G  D  T  V  Y   G  F  N  S  N
 841 ACCGGTCGTGACTTC CTCAGCACCACCAGC AACTCGCAGAAAGTG ATCTTTGCGGCCTGG
 281  T  G  R  D  F   L  S  T  T  S   N  S  Q  K  V   I  F  A  A  W
 901 GATGCGGGCGGCAAC GATACCTTCGACTTC TCCGGTTACACCGCT AACCAGCGCATCAAC
 301  D  A  G  G  N   D  T  F  D  F   S  G  Y  T  A   N  Q  R  I  N
 961 CTGAACGAGAAATCG TTCTCCGACGTGGGC GGCCTGAAGGGCAAC GTCTCGATCGCCGCC
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1021 GGTGTGACCATTGAG AACGCCATTGGCGGT TCCGGCAACGACGTG ATCGTCGGCAACGCG
 341  G  V  T  I  E   N  A  I  G  G   S  G  N  D  V   I  V  G  N  A
1081 GCCAACAACGTGCTG AAAGGCGGCGCGGGT AACGACGTGCTGTTC GGCGGCGGCGGGGCG
 361  A  N  N  V  L   K  G  G  A  G   N  D  V  L  F   G  G  G  G  A
1141 GATGAATTGTGGGGC GGTGCCGGCAAAGAC ATCTTCGTGTTCTCT GCCGCCAGCGATTCC
 381  D  E  L  W  G   G  A  G  K  D   I  F  V  F  S   A  A  S  D  S
1201 GCACCGGGCGCTTCA GACTGGATCCGCGAC TTCCAGAAGGGGATC GACAAGATCGACCTG
 401  A  P  G  A  S   D  W  I  R  D   F  Q  K  G  I   D  K  I  D  L
1261 TCGTTCTTCAATAAA GAAGCGCAGAGCAGC GATTTCATTCACTTC GTCGATCACTTCAGC
 421  S  F  F  N  K   E  A  Q  S  S   D  F  I  H  F   V  D  H  F  S
1321 GGCACGGCCGGTGAG GCGCTGCTGAGCTAC AACGCGTCCAGCAAC GTGACAGATTTGTCG
 441  G  T  A  G  E   A  L  L  S  Y   N  A  S  S  N   V  T  D  L  S
1381 GTGAACATCGGTGGG CATCAGGCGCCGGAC TTCCTGGTGAAAATC GTCGGCCAGGTAGAC
 461  V  N  I  G  G   H  Q  A  P  D   F  L  V  K  I   V  G  Q  V  D
1441 GTCGCCACTGACTTT ATCGTGTAA
 481  V  A  T  D  F   I  V  *
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Sequence analysis

A nucleotide sequence of 1500 bp could be read from the ladder sequence. The derived amino acid sequence showed single open reading frame of 1464 nucleotides (Fig. 2) encoding a protein of 487 amino acid residues (Fig. 4).

The sequence has an ATG codon at nucleotide position 1-3 and a termination codon at nucleotide position 1462-1464. The sequence was compared with the published sequence (Fig. 3), which showed homology of 100% with Serralysin metalloprotease gene of *Serratia marcescens*, strain 2170. The sequence was submitted to NCBI genbank nucleotide database and accession number (KP869847) was obtained. The GC content of the amplified gene was 58% and AT content was 42%. The derived amino acids from the sequenced nucleotides were found to be of 487 amino acids (Fig. 4) with the stop codon TAA at 1464 bp from ATG (start codon). The derived amino acid sequence has total of 56 acidic amino acids including 18 glutamic acid residues and 38 aspartic acid residues, and 29 basic amino acids including 10 arginine residues and 19 lysine, yielding a net negative charge on protein rendering the protein acidic.

In silico analysis of the conserved domains using databases available at:

http://www.ncbi.nlm.nih.gov/structure/cdd/wrpsb.cgi clearly showed that the serrapeptidase has a conserved domain

which is responsible for the zinc binding and contains the motif 192 HEIGHAL 199,240 GDNGGHY 247,321 LNEKSFSDVGG 331 and His- 192,196 residue and Glu- 323 at active site (Fig. 4), hence it belongs to zinc metalloprotease super family.

The similarities of these conserved domains with other proteases like thermolysin, Bacillus subtilis neutral protease, suggest that the derived amino acid sequence may be a metalloprotease. The new Genetic engineering approaches have helped to express gene of interest in heterologous host resulting in overproduction of the gene product.

Similarly cloning of Serrapeptidase gene in heterologous host will help in qualitatively and quantitively better protease production for further biochemical characterization.

References

Alexander, III, A.D., Eagle-Oden, G.S. 1999. The life and works of Dr.Hans Nieper: The Curious Man. Avery Publishing Group. Pp. 82.

Bayona, Sarah Jane, B., Chua, Lennie Lynn Y., Tan, Randall Isaac, F. 2008-2009. Unknown bacteria identification: The *Serratia marcescens* Project. Microbiology Laboratory, first Semester, A.Y.

Decedue, C.J., Broussard, E.A., Larsen, A., Braymer, H.D. 1979. Purification and characterization of Sertatia marcescans. *Biochem. Biophys. Acta*,. 569: 293–301.

Grimont, P.A.D., Grimont, F. 1984. Gene VIII. Serratia. In: Krieg, N.R., Holt, J.G. (Eds), Bergey's manual of systematic bacteriology, Vol. 1. Baltimore, Williams and Wilkins. Pp. 477–484.

Kodama, R., Nakasuji, Y., Yamauchi, S., Nishio, M. 1965. *J. Sericult. Sci. Japan*, 34: 206.

Matsumoto, D., Maeda, H., Talada, K., Kamata, R., Omamura, R. 1984. Purification and characterization of four proteases from a clinical isolate of *Serratia marcescens* kums 3958. *J. Bacteriol.,* 157: 225–232.

Miyata, K., Tomoda, K., Isono, M. 1971. Serratia protease part III, Characteristics of the enzyme as metalloenzyme. *Agr Biol Chem*., 35: 460–467.

Mohankumar, A., Hari Krishna Raj, R. 2011. Production and characterization of serratiopeptidase enzyme from *Serratia marcescens*. *Int. J. Biol.*, 3: 39–51.

Nakahama, K., Yoshimura, K., Marumoto, R., Lee, I.S., Hare, T., Matsubara, H. 1986. Cloning and sequencing of serratia protease gene. *Nucleic Acid Res.*, 14: 5843–5855.

Nakamura, S., Hashimoto, Y., Mikami, M., Yamanaka, E., Soma, T., Hino, M., *et al.* 2003. Effect of the proteolytic enzyme serrapeptase in patients with chronic airrway disease. *Respirology*, 8: 316–320.

Schmitz, G., Braun, 1985. V: Cell-bound proteases of *Serratia marcescens*. *J. Bacteriol.*, 161: 1002–1009.

Selvakumar, G., Mohan, M., Kundu, S., Gupta, A.D., Joshi, P., Nazim, S., Gupta, H.S. 2008. Cold tolerance and plant growth promation potential of *Serratia marcescens* strain SRM (MTCC 8708) isolated from flowers of summer sqash (*Cucurbita pepo*) *Lett. Appl. Microbiol.*, 46: 171–175.

Tao, K., Yu, X., Liu, Y., Shi, G., Liu, S., Hou, T. 2007. Cloning, expression, and purification of insecticidal protein Pr596 from locust pathogen *Serratia marcescens* HR-3. *Curr. Microbiol*., 55(3): 228–233.

Yamasaki, H., Tsuji, H., Siki, K. 1967. Anti-inflammatory action of a protease, TSP, produced by serratia. *Folia Pharmacol. Japan*, 18: 302–314.