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BIOCHEMICAL ANALYSIS AND HOMOLOGY MODELING OF COLLAGENASE PROTEIN OF *SCYLLA SERRATA CRAB*

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ABSTRACT

Scylla serrata (often called mud crab or mangrove crab, although both terms are highly ambiguous, as well as **black crab**) is an economically important species of crab found in the estuaries and mangroves of Africa, Australia and Asia. In their most common form, the shell colour varies from a deep, mottled green to very dark brown. The enzyme collagenase is extracted from Uca pugilator and it is estimated by using Lowry's method. Collagenase is biochemically analysed with SDS-PAGE and HPLC. The activity of the enzyme is assayed and characterized by determining the optimum pH and temperature. The effect of activator and inhibitor on collagenase activity is obtained. To analyse the primary sequence and to predict the homology modeling of collagenase protein in *Sylla serrata* using bioinformatics tools Like Prot param, Swiss model server. The modeled 3D structure of the peptide clearly shows the potential antigen binding sites which would be useful in structure based drug designing studies in future.

KEYWORDS: *Scylla serrata*, SDS –PAGE, HPLC, Prot Param, Swiss Model Server.

INTRODUCTION

Scylla serrata inhabits muddy bottoms, mangrove marshes and river mouths in estuarine environments. It is native to the Indo-Pacific and has been introduced to Florida, Hawaii and elsewhere, most often intentionally in attempts to establish populations of this commercially important species.

Scylla serrata from Karwar, India. Interest in the aquaculture of this species has been high due to the high demand/price for them, high flesh content and rapid growth rates in captivity. In addition, they have a high tolerance to both nitrate and ammonia (twice that of the similar sized Portunus pelagicus), which is beneficial because ammonia-N is often the most limiting factor on closed aquaculture systems. Their high ammonia-N tolerance may be attributed to various unique physiological responses which may have arisen due to their habitat preferences.

Studies indicate *Scylla serrata* become reproductively mature starting at around 90 mm carapace width, often within the first year of life (Robertson and Kruger 1994, Knuckey 1996).

Male crabs approach female crabs before the femeles have undergone a precopulatory molt, grasping them with their chelipeds and first pair of walking legs and carrying them around for up to several days until the females molt. On molting, males turn the females over and initiate copulation, delivering non-motile spermatozoa that may be retained by the females for up to several weeks to months before being used to fertilize multiple clutches of up to 2 million eggs each (Chen 1976). Hill (1996) notes that females bearing egg masses on their pleopods migrate offshore where the eggs hatch in a few weeks.

Enzymes are proteins that catalyze (*i.e.*, increase or decrease the rates of) chemical reactions. In enzymatic reactions, the molecules at the beginning of the process are called substrates, and they are converted into different molecules, called the products. Almost all processes in a biological cell need enzymes to occur at significant rates. Since enzymes are selective for their substrates and speed up only a few reactions from among many possibilities, the set of enzymes made in a cell determines which metabolic pathways occur in that cell.

Like all catalysts, enzymes work by lowering the enzyme activity for a reaction, thus dramatically increasing the rate of the reaction. As a result, products are formed faster and reactions reach their equilibrium state more rapidly. Most enzyme reaction rates are millions of times faster than those of comparable un-catalyzed reactions. As with all catalysts, enzymes are not consumed by the reactions they catalyze, nor do they alter the equilibrium of these reactions. However, enzymes do differ from most other catalysts by being much more specific. Enzymes are known to catalyze about 4,000 biochemical reactions. A

few RNA molecules called ribozymes also catalyze reactions, with an important example being some parts of the ribosome. Synthetic molecules called artificial enzymes also display enzyme-like catalysis.

Enzyme activity can be affected by other molecules. Inhibitors are molecules that decrease enzyme activity; activators are molecules that increase activity. Many drugs and poisons are enzyme inhibitors. Activity is also affected by temperature, chemical environment (*e.g.*, pH), and the concentration of substrate. Some enzymes are used commercially, for example, in the synthesis of antibiotics. In addition, some household products use enzymes to speed up biochemical reactions (*e.g.*, enzymes in biological washing powders break down protein or fat stains on clothes; enzymes in meat tenderizers break down proteins into smaller molecules, making the meat easier to chew).

In 1959, the first commercially available collagenase isolated from *Clostridium histolyticum* was offered by Worthington. At that time, only one preparation of crude enzyme was offered. Collagenases are endopeptidases that digest native collagen in the triple helix region. Collagens are the major fibrous component of animal extracellular connective tissue. Bacterial collagenases differ from vertebrate collagenases in that they exhibit broader substrate specificity (Peterkofsky 1982, Birkedal-Hansen 1987).

METHODS

The enzyme collagenase is extracted from the genus Uca. The amount of protein present in the sample is estimated by plotting OD value at 640nm against the standard protein using Lowry's method. The biochemical analysis of the enzyme is done by using Sodium Dodecyl Sulphate - Poly Acrylamide Gel Electrophoresis and High Performance Liquid Chromatography. The proteins get separated on the basis of molecular weights. When the electrophoretic run is completed the gel is immersed in 0.25% Coomassie blue solution for 30 minutes for staining. The gel is destained with 40% methanol and 10% acetic acid. The protein bands are observed clearly under UV- Trans illuminator. The mobile phase used in HPLC is 0.1% phosphoric acid and acetonitrile in the ratio 80:20. Sample is precipitated by 60% ammonium sulphate and the precipitate is recovered and purified further on G 200 sephadex. The sample is placed in auto sampler tray and run the HPLC. High pressure liquid chromatogram is obtained with the help of computer.

Using bioinformatics tools protein sequence was analyzed using PROTPARM tool and the secondary structure was predicated using GOR IV tool to identify the various structural regions present in the protein sequence. Finally the collagenase protein was modeled through swiss model server and validated using rapper server.

RESULTS

As with all forms of gel electrophoresis, molecules may be run in their native state, preserving the molecules' higher-order structure or a chemical denaturant may be added to remove this structure and turn the molecule into an unstructured linear chain whose mobility depends only on its length and mass-to-charge ratio. In most proteins, the binding of SDS to the polypeptide chain imparts an even distribution of charge per unit mass, thereby resulting in a fractionation by approximate size during electrophoresis. The result of SDS-PAGE is shown in Fig 1.

High-performance liquid chromatography is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out the column. The chromatogram obtained for standard and sample collagenase is given in Fig 2 and 3.

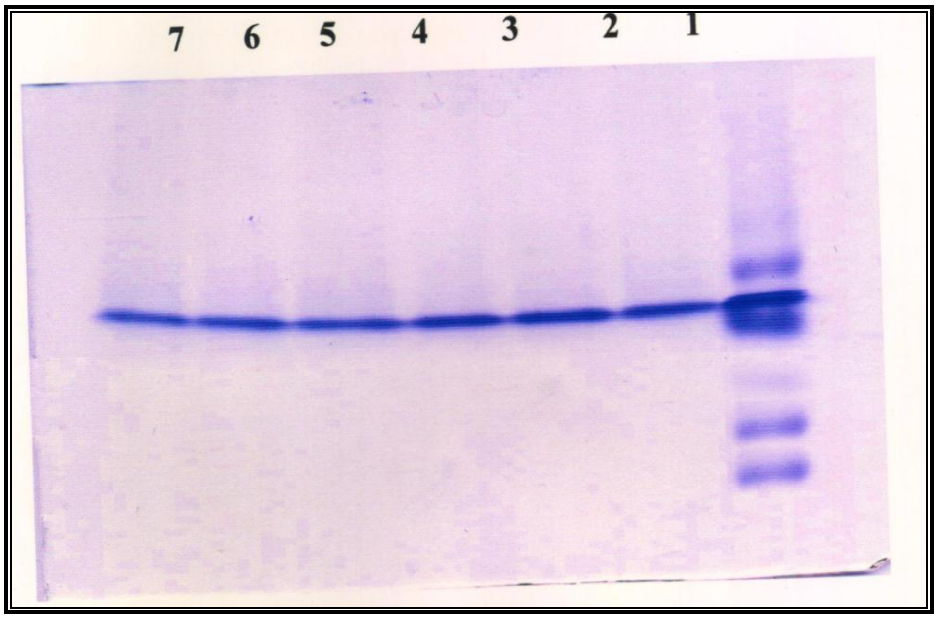

Figure 1 .SDS-PAGE.

Lane: 1 Protein Marler (66, 000, 43, 000, 29, 000, 14,000Da)

Lane: 2 Crude enzymes

Lane: 3 Supernatant obtained from centrifugation.

Lane: 4 Extract obtained from Ion exchange chromatography

Lane: (5-7) Extract obtained from after analysis

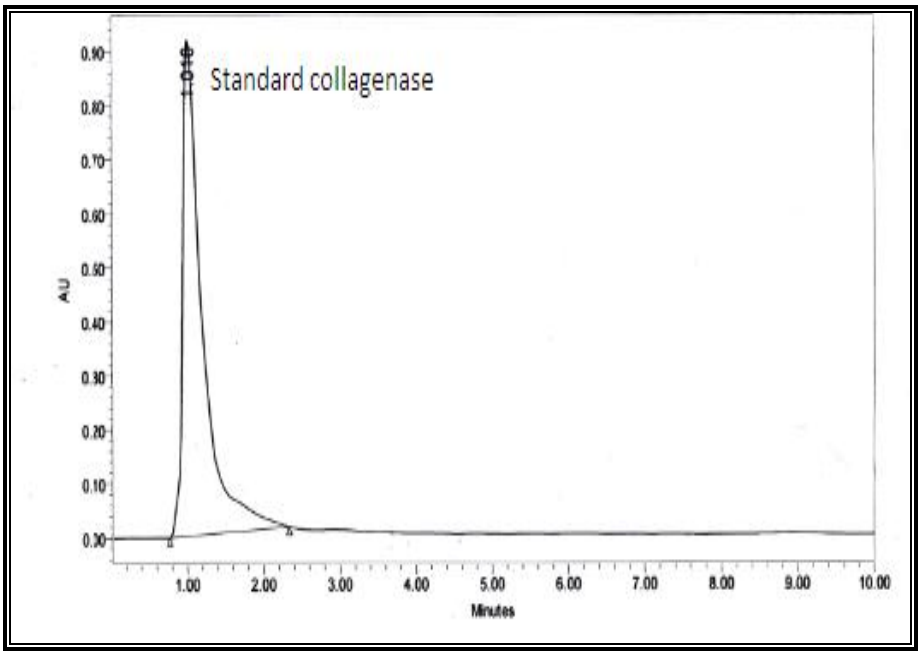

Fig 2: HPLC chromatogram of standard collagenase.

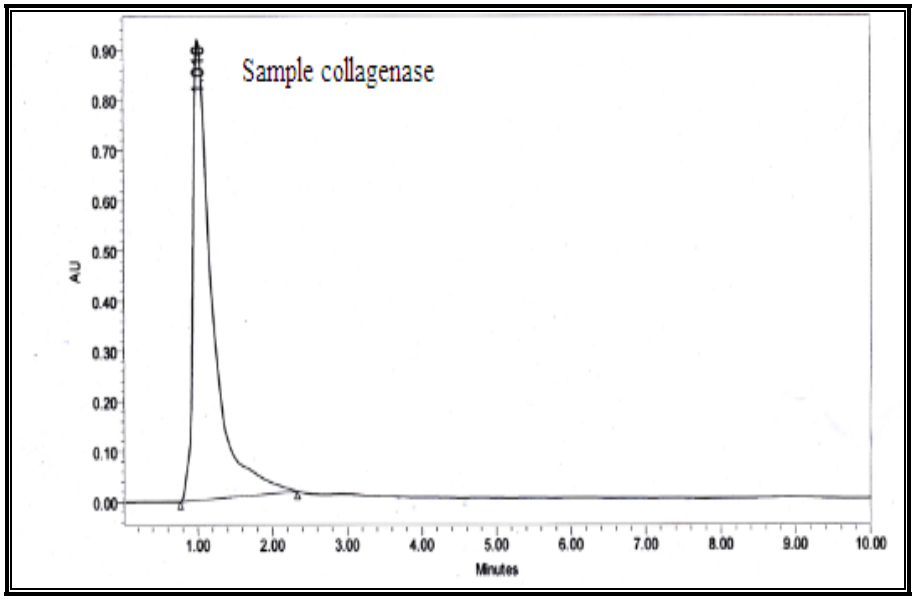

Fig 3: HPLC chromatogram of sample collagenase.

BIOINFORMATICS

Using bioinformatics tools protein sequence was analyzed using PROTPARM tool and the secondary structure was predicated using GOR IV tool to identify the various structural regions present in the protein sequence. Finally the collagenase protein was modeled through swiss model server and validated using rapper server.

Protein sequence retrieval system

>AAC47030.1 serine collagenase 1 precursor [Scylla serrata]

MIVKLALILVCVALASGNPAAGTEWRWKSPKPLMTPIGPVKSSRIVGGVEAVPNSWP
HQAALFIDDMYFC

GGSLISPEWILTAAHCMDGAGFVDVVLGAHNIREDEATQVTIQSTDFTVHENYNSFVI
SNDIAVIRLPSP

VTLTAAIATVGLPSTDVGVGTVVTPTGWGLPSDSALGISDVLRQVDVPIMSNADCDA
VYGIVTDGNICID

STGGKGTCNGDSGGPLNYNGLTYGITSFGAAAGCEAGYPDAFTRVTYFLDWIQTQT
GITP

Fasta format sequence of collagenase protein.

SECONDARY STRCTURE ANALYSIS

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        10        20        30        40        50        60        70
         |         |         |         |         |         |         |
MIVKLALILVCVALASGNPAAGTEWRWKSPKPLMTPIGPVKSSRIVGGVEAVPNSWPHQAALFIDDMYFC
cccceeeeeeeeccccccccccceeeeccccceecccccccccceeceeeeccccccceeeeeeeeeeec
GGSLISPEWILTAAHCMDGAGFVDVVLGAHNIREDEATQVTIQSTDFTVHENYNSFVISNDIAVIRLPSP
eeeeeccceeeeeeccccccceeeeeeccccchhhhhheeeeccceeecccccceeecccceeeecccc
VTLTAAIATVGLPSTDVGVGTVVTPTGWGLPSDSALGISDVLRQVDVPIMSNADCDAVYGIVTDGNICID
cchhhhhhhcccccccceeeeeecccccccccccchhhheeecceeeecccccceeeeeecccceeee
STGGKGTCNGDSGGPLNYNGLTYGITSFGAAAGCEAGYPDAFTRVTYFLDWIQTQTGITP
eecccceecccccccccccceeeeeeecccccccccccceeeeecccceeeeceeeec
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Sequence length :   270
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GOR4 :
   Alpha helix     (Hh) :    17 is   6.30%
   3₁₀ helix       (Gg) :     0 is   0.00%
   Pi helix        (Ii) :     0 is   0.00%
   Beta bridge     (Bb) :     0 is   0.00%
   Extended strand (Ee) :   110 is  40.74%
   Beta turn       (Tt) :     0 is   0.00%
   Bend region     (Ss) :     0 is   0.00%
   Random coil     (Cc) :   143 is  52.96%
   Ambiguous states (?)  :     0 is   0.00%
   Other states          :     0 is   0.00%
```

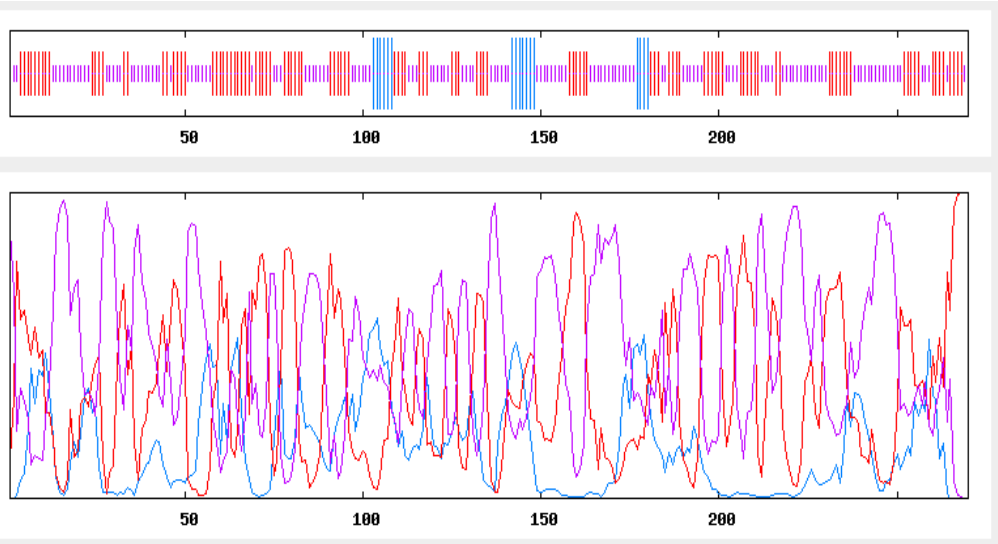

Fig 4: Blue – Helix, Red – sheets and Blue –Coils regions of the Collagenase protein sequence.

Homology modeling

The modelled protein 3D strucure were viewed with the help of advanced molecular viusalization software called discovery studio .Inorder to identyfy the structural region and classify the entire 3d structural elements.

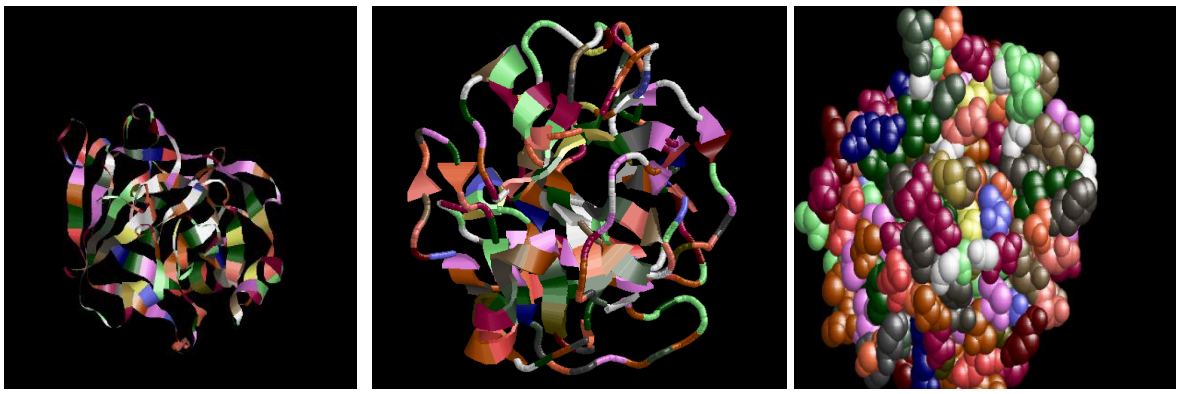

Fig 5: The above picture represents the ribbon and cartoon, space fill Model of collagenase protein.

CONCLUSION

From the above study it is concluded that *Scylla serrata* can be used as suitable source for the production of collagenase. This can be used as an effective, selective method of removing necrotic tissue from pressure ulcers, leg ulcers and burns. Clinicians often combine enzymatic deriding agents with other methods of debridement such as surgical debridement, autolysis, and conservative sharp would debridement. A typical approach might be initial surgical.

Collagenase is selective to necrotic tissue but is not harmful to clean tissue when applied to the wound bed. It may be applied directly to the wound bed, or if the tissue is slick, it may be easier to apply to the dressing. This collagenase would be very useful for medicine, food industry and leather scrap recycles.

The primary aim of the present bioinformatics work is to analyze the complete molecular protein profiling and secondary structure prediction of the *(Scylla serrata)* collagenase protein sequence. Based on the results of primary and secondary structure prediction, the results show physiochemical Properties (Mw, pI, molecular weight, total number of Atoms etc,). The results of secondary structure show the Total percentage of helix, sheets and coils present in the collagenase protein sequence. From the results of homology studies and evaluation of collagenase shows the Number of residues in favoured region 93.2%. All the above results will be useful for pharmacology and Pharmacogenomics studies.

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