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BIOPHYSICAL CHARACTERIZATION AND FUNCTIONAL ANNOTATION OF ENOLASE PROTEIN IN *ANUSTRCA ANNLIPES*

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ABSTRACT: *Anustrca annlipes (fiddler crab)*, often called crab, are decaped crustaceans of the uca family. They belong to the ocypodidae family, which contains over a hundred species of semi-terrestrial sea crabs. 11 of the 13 genera in the ocypodidae crab family are made up of more than 100 fiddler crab species. The species can be found on sea and brackish intertidal mud flats, lagoons and swamps, mangroves, salt marshes, and sandy or muddy west coasts. *Anustrca annlipes* also promotes the turnover and mineralization of critical nutrients and several species-specific body colour patterns that can act as intra- and intra-specific signals. Compared to female and smaller male uca annulipes crabs, they excavate their tunnels. An antimicrobial is a substance that kills or inhibits the development of bacteria. Antimicrobial medications are classified based on the bacteria they predominantly target. Through review number of antimicrobial proteins from *Anustrca annlipes* were identified. So the antimicrobial protein enolase from *Anustrca annlipes* sequence was retrieved from NCBI databases. Gene annotation was carried out by using amigo tools. Gene expression analysis done by using Jcat server. Sequential and functional analyses were carried out through online bioinformatics software tools.

INTRODUCTION: Crabs are infraorder Brachyura decapod crustaceans with a very short projecting "tail" (abdomen) (Greek: romanized: brachys = short, tail), which is normally covered fully under the thorax [1]. They can be found in all of the world's oceans, freshwater, and on land. They have a robust exoskeleton and a single pair of pincers [2]. Crabs have a thick exoskeleton made mostly of highly mineralized chitin and are armed with a pair of chelae (claws).

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Crabs range in size from the pea crab, which is only a few millimetres broad, to the Japanese spider crab, which has a leg span of up to 4 meters (13 ft) [4-5]. Other crustaceans with similar appearances, such as king crabs and porcelain crabs, are not actual crabs, but have evolved traits similar to true crabs through a process known as carcinisation [6]. Crabs prefer to dwell near water, particularly saltwater or brackish water. They can be found in every ocean on the planet [7-8]. Some dwell entirely in the water, while others live near the water's edge, in and among the rocks or sand along the coasts.

Description: Crabs have a thick exoskeleton made mostly of highly mineralized chitin and are armed with a pair of chelae (claws). Crabs range in size

from the pea crab, which is only a few millimeters broad, to the Japanese spider crab, which has a leg span of up to 4 meters (13 ft) [9]. Several other crustaceans with similar appearances, such as king crabs and porcelain crabs, are not true crabs but have evolved traits similar to actual crabs through a process known as carcinisation [10].

Habitat: *Leptuca annulipes* can be found in estuarine and marine environments and on protected beaches with sandy or rocky substrates [11]. It is most usually seen around marshes and along the banks of tidal streams.

Environment: Crabs are found in all of the world's oceans, as well as in fresh water and on land, particularly in tropical regions [12-15]. About 850 species are freshwater crabs Enolase protein: Enolase is a multifunctional protein that functions as a glycolytic enzyme in the cytoplasm of prokaryotic and eukaryotic cells [16]. -enolase is found on the surface of many different cell types, where it functions as a plasminogen receptor, concentrating proteolytic plasmin activity on the cell surface [17- 20]. -enolase appears to have other cellular functions and subcellular localizations separate from its well-established glycolytic enzyme and plasminogen receptor functions. Furthermore, the differential-enolase expression has been linked to a variety of diseases, including cancer, Alzheimer's disease, and rheumatoid arthritis, among others. -enolase has been found as a plasminogen receptor in a variety of cell types [21]. We focused on its significance in myogenesis as an example of an extracellular remodelling mechanism. We discovered that -enolase is expressed on the cell surface of developing myocytes and that -enolase/plasminogen binding inhibitors hinder myogenic fusion in vitro and skeletal muscle regeneration in mice [22 - 25]. Enolase could be regarded as a marker of pathological stress in a wide range of disorders, as it performs several of its several roles, most notably as a plasminogen receptor. This research focuses on the many roles of the -enolase/plasminogen axis in various diseases.

MATERIALS AND METHOD: The target protein and the nucleotide sequence of the enolase protein were obtained from the NCBI database. The obtained sequence is run through various bioinformatics tools listed below for sequence and functional analysis of enolase from *Anustrca annlipes*. The obtained sequence is submitted to the AmiGO program to learn about the annotation of the enolase protein. The annotated sequence from AmiGO is utilized to identify the JCAT server for Gene expression analysis. Bioedit was used for the sequence analysis of the enolase protein in *Anustrca annlipes*.

RESULTS AND DISCUSSION:

NCBI:

Protein Sequence: >XP_006370245.enolase-phosphatase E1 [*Anustrca annlipes*]

MAAAPPAVAVNGGGMAAAKVASQAYLESK
AVKDTRVLIADLCKQFYTLGWVSGTGGSITIK
AHDDSIPKRQQLILMSPSGVQKERMEPEDMY
VLATNGSILSSPSPKPYPYKPPKCSDCAPLFLK
AYDMRNAGAVIHSHGMESCLVTMINPLSKEF
RITHMEMIKGIQGHGYYDELVVPIIENTAYEN
ELTDSLAKAIEAYPKTTAVLVRNHGIYIWGDS
WISAKTQAECYHYLFDAAIKLHQIGLDWSTP
NHGPIQNVKVKAGMNNSNNRIEPLPRCIVLDI
EGTTTPITFVADVLFPYARDNVGRHLSATYDT
AETKDDINLLRTQVEDDLAQGVDGAIPIPTDD
AGKEEVIAALVANVEAMIKADRKITALKQLQ
GHIWRTGYENNELEGVVYDDVPEALEKWHA
LGIKVYIYSSGSRLAQRLIFGKTNYGDLRKYL
SGFFDTTVGNKKETRSYIEISESLGVDKPSDIL
FVTDVFQEAFAAKGAGL
DVMISIRPGNAPLPENHGFKTITSFAEI

Nucleotide Sequence: >KJ132918.1 *Anustrca annlipes* enolase gene, partial cds
GCTATGCGTATGGGAAGTGAGGTGTACCAT
CACCTGAAGGCTGTCATCAAGGGGCGCTTT
GGCCTTGATGCCACTGCTGTGGGTGATGAG
GGTGGCTTTGCCCCCAACATTCTGAACAAC
AAGGATGCTCTCCAGCTCATCCAGGAGGCC
ATCAACAAGGCTGGCTACACAGGCAAGATT
GAAATTGGAATGGATGTGGCTGCCTCTGAG
TTCTACAAAGGCAACAATGTCTATGAYCTT
GACTTCAAGACTGCTAACAATGATGGCTCC
CAGAAGATCT
CTGGTGACCAGCTCAGGGACATGTACATGG
AATTCTGCAAAGAGTTCCCC

The above result shows the fasta format of protein and nucleotide sequence of enolase protein in *Anustrca annlipes*

AMIGO TOOL:

TABLE 1: THE ABOVE TABLE SHOW THE GENE ANNOTATION RESULTS OF ENOLASE PROTEIN IN ANUSTRCA ANNLIPES

S. no.	Name of The Gene	Organism
1.	Enolase	*Austruca Annlipes*
2.	Enolase-phosphatase	Eremothecium gossypii ATCC 10895
3.	Enolase pthr	Populus trichocarpa
4.	Enolase-phosphatase	Tribolium castaneum

JCAT SERVER:

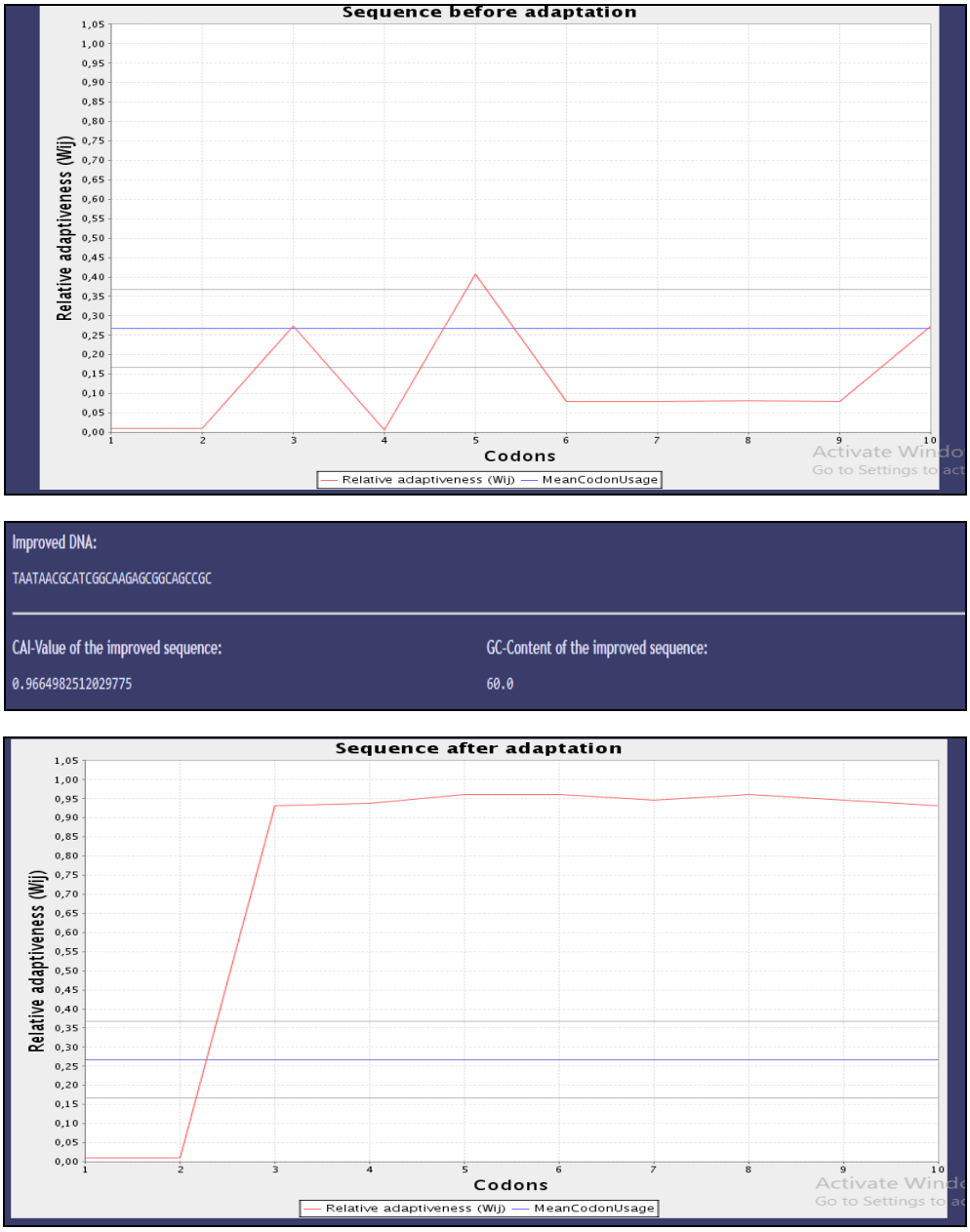


FIG. 1: THE ABOVE RESULTS SHOW THE GENE EXPRESSION ANALYSIS OF ENOLASE PROTEIN IN *ANUSTRCA ANNLIPES*

TABLE 2: THE ABOVE TABLE SHOWS THE GENE EXPRESION ANALYSIS OF TARGET SEQUENCE

S. no.	Name of the gene	Organisum	CAI (value)
1.	Enolase	*Austruca annlipes*	0.9664982512029775
2.	Enolase-phosphatase	*Eremothecium gossypii*	0.9733577397245378

BIOEDIT:

```
Protein: AXS63529.1 enolase, partial [Austruca annulipes]
Length = 134 amino acids
Molecular Weight = 14277.10 Daltons

Amino Acid   Number    Mol%
  Ala   A       15      11.19
  Cys   C        1       0.75
  Asp   D        9       6.72
  Glu   E       10       7.46
  Phe   F        6       4.48
  Gly   G       13       9.70
  His   H        4       2.99
  Ile   I        7       5.22
  Lys   K        8       5.97
  Leu   L        9       6.72
  Met   M        5       3.73
  Asn   N        9       6.72
  Pro   P        4       2.99
  Gln   Q        4       2.99
  Arg   R        3       2.24
  Ser   S        9       6.72
  Thr   T        6       4.48
  Val   V        5       3.73
  Trp   W        0       0.00
  Tyr   Y        5       3.73
```

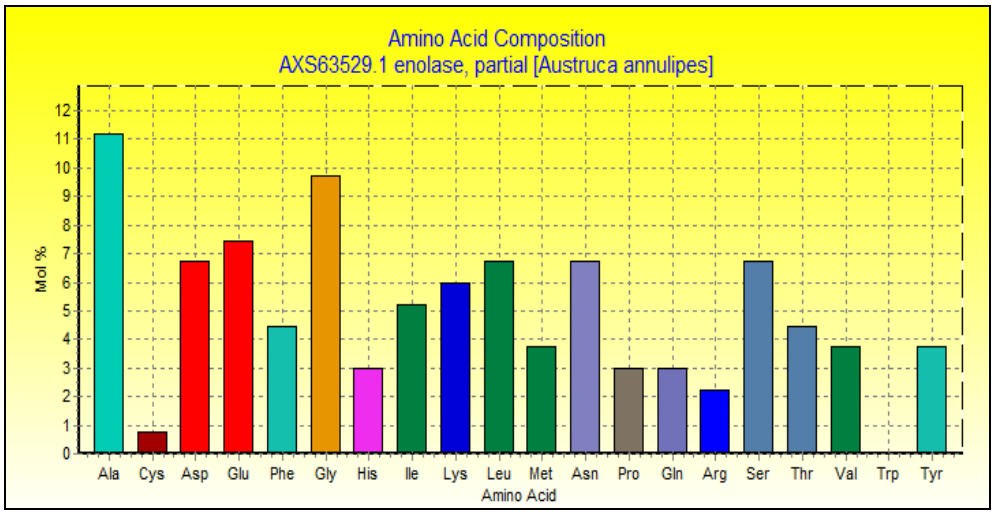
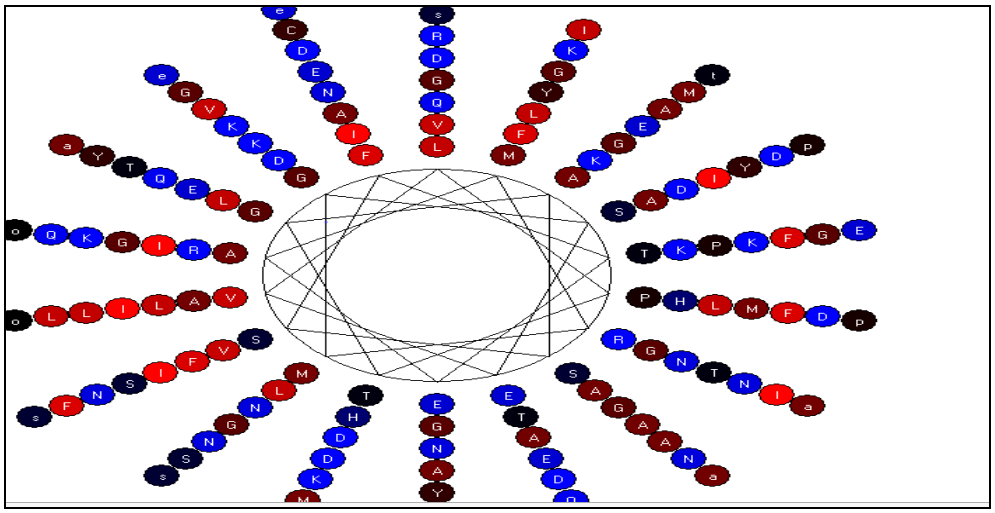


FIG. 2: THE ABOVE RESULT SHOWS THE AMINO ACID COMPOSITION OF ENOLASE PROTEIN IN *ANUSTRUCA ANNLIPES*



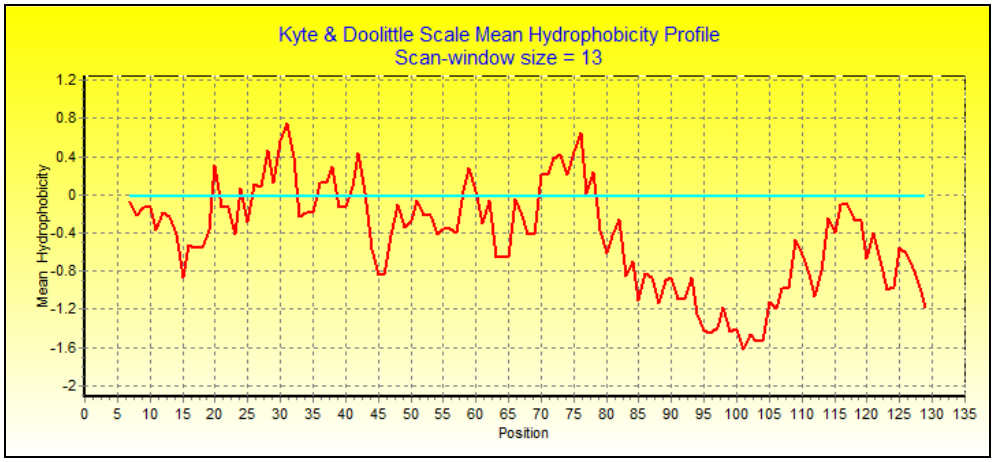


FIG. 3: THE ABOVE RESULTS SHOW THE HELICAL WHEEL STRUCTURE, KYTE AND DOLITTLE HYDROPHOBICITY PROFILE OF ENOLASE PROTEIN IN *ANUSTRCA ANNLIPES*

Nucleotide:

DNA Molecule: MG023250.1 Austruca annulipes isolate Uan_8 enolase gene, partial cds. Length = 366 base pairs. Molecular Weight = 111317.00 Daltons, single stranded. Molecular Weight = 222650.00 Daltons, double stranded. G+C content = 49.73%. A+T content = 46.45%

Nucleotide	Number	Mol%
A	90	24.59
C	88	24.04
G	94	25.68
T	80	21.86
S	3	0.82
H	2	0.55
N	9	2.46

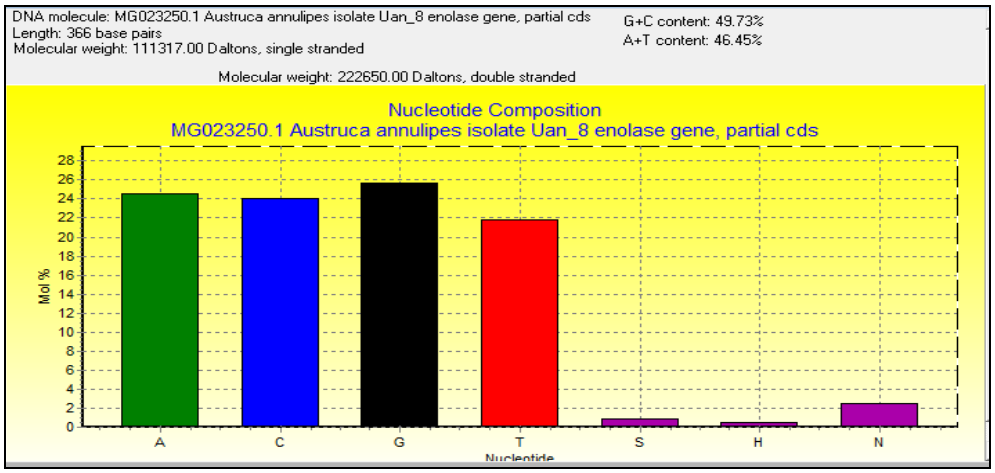


FIG. 4: THE ABOVE RESULT SHOW IS THE NUCLEOTIDE COMPOSITION OF ENOLASE PROTEIN IN *ANUSTRUCA ANNLIPES*

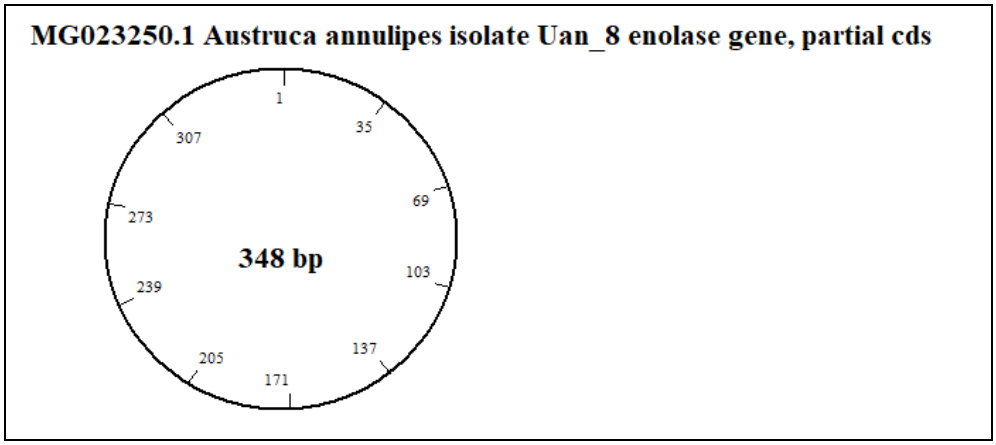


FIG. 5: THE ABOVE RESULT SHOW THE PLASMID STRUCTURE OF ENOLASE PROTEIN IN *ANUSTRCA ANNLIPES*

CONCLUSION: Enolase, also known as phosphopyruvate hydratase, is a cytosolic protein that is abundant in many species. Extracellular proteolysis is essential in many biological processes in multicellular organisms that need a dynamic rearrangement of cell-cell and cell-matrix connections, with the plasminogen activation (PA) system being one of the most important extracellular proteases. Furthermore, differential -enolase expression has been associated to a number of illnesses, including cancer, Alzheimer's disease, and rheumatoid arthritis. -enolase was found on the cell surface of developing myocytes, and -enolase/plasminogen binding inhibitors hinder in vitro myogenic fusion and skeletal muscle regeneration in mice.- enolase may be used as a marker of pathological stress in various diseases since it has multiple functions, the most important of which is as a plasminogen receptor. Ncbi databases were used to extract the antimicrobial protein enolase sequence from *Anustrca annlipes*. The amigo tools were used to annotate genes. Jcat server was used for gene expression analysis. Finally, sequence analysis was performed using bio edit tools. A detailed examination of protein will provide new information.

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