Phylogenetic Analysis of Lysozyme C from the Scorpion Mesobuthus eupeus Venom Gland

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Abstract: Many studies have been carried out on peptides and genes encoding scorpion toxins from the venom of the scorpion Mesobuthus eupeus. The scorpion venom contains a diversity of bioactive peptides which could cause toxic effects and can be candidates for drug design and development. The anti-microbial lysozymes among them are of great value. Lysozymes are hydrolytic enzymes characterized by the ability to cleave the β-(1, 4)-glycosidic bond between N-acetylmuramic acid and N-acetyl-D-glucosamine in a peptidoglycan layer, the major bacterial cell wall polymer. The total RNA was extracted from venom glands of Mesobuthus eupeus species of Khuzezan. cDNA was synthesized with extracted total RNA as template and modified oligo (dT) as primer. In order to amplify cDNA encoding a Lys-C peptide, semi-nested RT-PCR was performed with the specific primers followed by sequencing of the amplified fragment. The full-length cDNA sequence contains a 438 nucleotide open reading frame encoding a peptide of 144 amino acids with molecular weight of 16,702 kDa. A putative 22-residue signal peptide was identified. Based on the phylogenetic tree of MesoLys-C and α-type lysozyme of East Mediterranean M. eupeus it is concluded that M. eupeus of Khuzezan and East Mediterranean M. eupeus belong to different subspecies.

Key words: Phylogenetic analysis, anti-microbial protein, lysozyme C, scorpion venom, amino acids, Iran

INTRODUCTION

All known scorpion species possess a venom apparatus which has been an important determinant in contributing to the successful survival of these animals for >400 million years. Scorpion venom is a combinatorial library of peptides and proteins which could cause toxicological responses and can be candidates for drug design and development (Menez, 1998). Several recent studies have demonstrated that scorpine-like peptides isolated from the venomous gland of some scorpion species have anti-bacterial and -malaria effects (Conde et al., 2000). These and other anti-microbial peptides found in scorpions may serve as a promising lead candidate in the development of novel antibiotic molecules. In this context, lysozymes are of great importance. Lysozymes are muramidases that damage the peptidoglycan layer of the bacterial cell wall by hydrolysing β-(1, 4)-glycosidic linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues (Gandhe et al., 2007). The known lysozymes within the animal phyla are generally classified into 3 main types; chicken-type (α-type), invertebrate-type (i-type) and goose-type (g-type) (Bachali et al., 2002). The α-type lysozyme has been found in many organisms including viruses, bacteria, plants, insects, reptiles, birds and mammals (Tamura et al., 2007) including scorpions (Elgar et al., 2006). Generally, lysozymes play an important defense role in the innate immunity. However, the exact biological role of lysozymes from scorpion venoms remains to be explored as they have a relatively high expression level. In this research, researchers report the characterization and phylogenetic analysis of α-type lysozyme from the venom glands of Mesobuthus eupeus scorpions of Buthidae family which are widespread in Iran, especially in Khuzezan province.

MATERIALS AND METHODS

Scorpion samples: The specimens of M. eupeus were collected in Khuzezan province (Iran) and transported to the Reference Laboratory of the Razi Institute. They were killed 2 days after manual extraction of the venom to allow the toxin producing cells of the venom glands to enter into a secretory phase. Twenty separated venom glands were used for total RNA extraction.

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**Total RNA extraction:** About 4 μg of total RNA was extracted from the venom glands of scorpions (0.5 g of tissue material) using RNA™ (Cinagene, Iran) according to the manufacture procedure. The RNA pellets were dissolved in DEPC-ddH₂O and used for cDNA synthesis immediately.

**cDNA library synthesis:** The cDNA was synthesized from the extracted total RNA as template and modT (modified oligo-dT) (5'-ggtctgactgctgatctactttttttttttttt) as primer. ModT was added to the extracted RNA and incubated at 70°C for 5 min and immediately transferred into ice for 2 min. The mixture of 5× buffer, dNTPs, ribolock, reverse transcriptase, and ddH₂O was added to the samples followed by incubation at 42°C for 60 min after which the samples were incubated at 70°C for 10 min and immediately transferred into ice.

**Semi-nested RT-PCR amplification:** For the cDNA amplification, semi-nested RT-PCR technique was used. The 1st round of PCR was performed using modT-R (5'-ccagactgctgatctggttt-3'), lys-F 5'-geggtat ccagatgtctgactttttttttttttttttc primers and synthesized cDNA as template. The 2nd round of PCR was performed using lys-F and lys-R (5'-geggtatccagactggttttgattgataattgtttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttt
said that *M. eupeus* of Khuzestan and East Mediterranean *M. eupeus* belong to different subspecies.

**ACKNOWLEDGEMENTS**

This research was supported by the Razi Reference Laboratory of Scorpion Research (RRLS), Iran. Researchers are grateful to Dr. Ghannamghami, Mr. Taghavi, Mr. Maslhipour and Mr. Bahrami for their kind supports.

**REFERENCES**


