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Short Comm.

ISOLATION AND CHARACTERIZATION OF TROPOMYOSIN FROM *MACROBRACHIUM ROSENBERGII* (GIANT FRESHWATER PRAWN)

Zailatul Hani Mohamad Yadzir[1*], Rosmilah Misnan[2], Faizal Bakhtiar[1], Noormalin Abdullah[1] and Shahnaz Murad[1]

[1]Allergy and Immunology Research Centre, Institute for Medical Research, Jalan Pahang, 50588, Kuala Lumpur, Malaysia

[2]Department of Biology, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900 Tanjong Malim, Perak, Malaysia

Email: zailatul@imr.gov.my (**Corresponding Author*)

Abstract: The major prawn allergen has been shown to be a 34 to 38 kDa heat-stable protein known as tropomyosin. Tropomyosin is associated with thin filaments in muscle and plays a role in regulating muscle contraction. Our phase one study on the characterization of *Macrobrachium rosenbergii* (giant freshwater prawn) allergens by using 2-dimensional electrophoresis and mass-spectrometry analysis has also identified tropomyosin as the major allergen of this prawn. The purpose of this study was to isolate and characterize tropomyosin from the giant freshwater prawn, *Macrobrachium rosenbergii*. Tropomyosin was isolated from the total RNA obtained from prawn muscle using RT-PCR (Reverse Transcriptase-Polymerase Chain Reaction) method followed by sequencing. The partial tropomyosin fragment of ~800 bp has been isolated and sequenced. Future studies will focus on cloning and expression of the full-length of tropomyosin in order to produce recombinant tropomyosin protein of this species of prawn and subsequently further characterize its biochemical and immunological properties.

Keywords: Tropomyosin, major allergen, giant freshwater prawn, *Macrobrachium rosenbergii*, prawn allergy.

Findings

Allergy is usually diagnosed either *in vivo* by the skin prick test (SPT) or *in vitro* by measuring allergen-specific IgE using natural allergen extracts, containing a variety of allergenic and non-allergenic components that make them too complex to be standardize [1]. The lack of standardization of natural allergen extracts for allergy test, mainly because of the lost of the allergenic activity during the extraction procedure and storage, results in low diagnostic accuracy [1,2]. Another concern is that natural extract immunotherapy risks inducing allergen-specific IgE antibodies to which the patient was not previously sensitized [3,4].

Many of the problems associated with using natural extracts for allergy diagnosis and treatment may be overcome with recombinant allergens. High purity recombinant allergens

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may be produced by using controlled production procedures that yield defined molecules with known molecular, immunologic and biological characteristics [5]. Furthermore, they may be modified to reduce their allergenic activity and to foster certain advantageous immunologic properties [5,6]. Recombinant allergens equalling the natural allergens are available for diagnostic and therapeutic purposes, and modified versions have been developed to reduce IgE-mediated side effects during immunotherapy [6].

Shellfish is known to be a common cause of food hypersensitivity reactions. Among shellfish allergies, prawn is the most frequent culprit [7,8]. In sensitized individuals, prawn allergens can provoke allergic symptoms including urticaria, angioedema, laryngospasm, asthma and life-threatening anaphylaxis [9,10]. For many years, reports on the identification of prawn allergens were limited to the family *Penaeidae* (seawater prawn). There are very few reports on the identification and molecular characterization of allergen in *Macrobrachium rosenbergii*, also known as the giant freshwater prawn or Malaysian freshwater prawn. Our phase one study on the characterization of giant freshwater prawn allergens by using 2-dimensional electrophoresis and mass-spectrometry analysis has identified tropomyosin as the major allergen of this prawn [11]. So the purpose of this study was to isolate and characterize tropomyosin from the giant freshwater prawn, *Macrobrachium rosenbergii*.

Live *Macrobrachium rosenbergii* (giant freshwater prawn) was purchased from a freshwater prawn pond at Rawang, Selangor, Malaysia. Total RNA was extracted from the muscle using TRIzol (Invitrogen, Germany) according to the manufacturer's instructions. Reverse transcription (RT) - Polymerase Chain Reaction (PCR) was performed using the HelixCript One-Step RT-PCR Kit with HelixAmp *Hot Taq* (NanoHelix, South Korea) by using the pairs of specific primers (5'-cag-gcg-atg-aag-ctg-gag-aag-3' and 5'-tta-gta-gcc-aga-cag-ttc-gct-ga-3'). 1.0% agarose gel electrophoresis was used for detection of PCR products. The bands on the gel were visualized on an ultraviolet light transilluminator and photographed using the Bio Imaging System in Gene Snap Program (Syngene,UK). The sequencing was carried out by First Base Laboratories Sdn Bhd, Malaysia. The sequences were compared with GenBank using the BLAST program to identify sequences with high homology.

Based on gel electrophoresis analysis, ~800 bp band was seen as shown in Figure 1. The PCR product then was sequenced and revealed that this protein consists of 767 nucleotides (Figure 2). The GenBank BLAST search for the sequence showed high homology to tropomyosin protein from various prawn species as shown in Table 1.

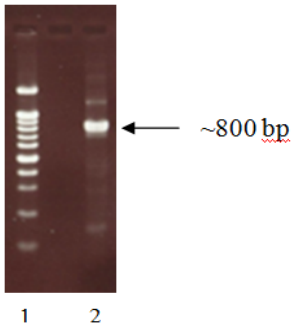

Figure 1. Detection of PCR product. Lane 1: Ladder marker. Lane 2: PCR products ~800 bp.

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agggcggataccttggaacagcagaacaaggaggccaacatcagagctga 50
gaaggccgaggaggaggttcacaaccttcagaagcggatgcaacaacttg 100
agaacgaccttgatcaggtgcaggaatcgctactgaaggctaacacccag 150
ctagaagagaaggacaaggctctgtctaatgctgagggtgaagtggctgc 200
acttaaccgtcgcatccagctgctagaggaggatctggaacgctcagagg 250
agcgccttaacacagccaccacaaaactggcagaggcttcccaggccgcc 300
gacgagtctgagcgcatgcgcaaggtgctggagaatcgttccctgtcaga 350
tgaggagcgtatggatgctctagagaaccagcttaaagaggcccgattct 400
tggctgaggaagcagacaggaaatacgacgaggttgcccgtaagctggcc 450
atggttgaggctgatcttgagcgagctgaggagcgtgcagaaactggtga 500
atctaagattgttgaacttgaggaggagctgcgtgttgttggcaacaact 550
tgaagtctcttgaggtgtctgaggagaaggccaaccagcgtgaggaggct 600
tacaaggagcagatcaagaccctagccaacaagctgaaggcggctgaggc 650
tcgggctgagttcgccgagaggtctgtgcagaagctccagaaggaggtcg 700
acaggcttgaagacgaactggttaacgaaaaggagaagtacaagtccatt 750
accgacgagcgggcctt                                   767
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Figure 2. Nucleotide sequences of the 767 bp of tropomyosin.

Table 1. Protein analysis of the sequence of tropomyosin.

Organisms	Accession Number	Blast Score	Nucleotide Identities (%)
Penaeus monodon	HM034312.1	575	99%
Litopenaeus vannamei	EU410072.1	563	98%
Metapenaeus ensis	U08008.1	547	98%

So far, tropomyosin has been established as the major allergen of a number of prawns, termed *Pen i* 1, *Pen a* 1 and *Met e* 1 depending on the species used [12-14]. Tropomyosins are proteins that play a role in the contractile activity of muscle cells [15]. In light of the

successful application of molecular cloning in the identification of prawn allergens has led to the production of recombinant prawn allergens. Recombinant *Met e* 1 from *Metapenaeus ensis*, is a recombinant tropomyosin [14]. Another well characterized recombinant tropomyosin is rPen *a* 1 from *Penaeus aztecus* [16]. Our study agrees with other reports identifying tropomyosin as the common prawn allergen.

We report the isolation and characterization of a *Macrobrachium rosenbergii* (giant freshwater prawn) tropomyosin protein. In the future, we will focus on the cloning and expression of the full-length of tropomyosin in order to produce recombinant tropomyosin protein from this species of prawn to further define its biochemical and immunological properties. These findings may contribute directly to the advancements in diagnosis, management of allergic patients via the development of immunotherapy and to the standardization of allergenic test products as tools in molecular allergology.

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