Amplification of Tropomyosin from Freshwater prawn (*Macrobrachium rosenbergii*)

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Abstract

Food allergies are a major health problem and are caused by an adverse immune response to food allergens. Such allergens are usually proteins that react with IgE antibodies to induce an allergic reaction. Among food allergens, shellfish are a frequent cause of adverse food reactions. Shellfish include crustaceans such as crab, shrimp and crayfish, and mollusca such as squid, octopus, oyster and mussel. They are common ingredients in many dishes. One of the major allergens in crustaceans has been identified to be a heat stable muscle protein, tropomyosin. Tropomyosin is a cross-reacting allergen among invertebrates and is assumed to be the major allergen common among decapod crustaceans. However, sequence of the tropomyosin from the freshwater prawn, *Macrobrachium rosenbergii* is not yet available. The present work reports a successful PCR amplification of a tropomyosin cDNA fragment of 900bp which will be sequenced in the future. In addition, our sturdies in the near future will include, cloning of a full-length gene of tropomyosin from *M. rosenbergii* and characterization of the protein for understanding their evolution.

Introduction

A food allergy is an immunological response to a food protein. The reaction is an IgE mediated type I hypersensitive reaction. When a protein or immunologically active protein fragment crosses the mucosal membrane surfaces, and is processed by macrophages and lymphocytes, stimulation of the production of protein-specific IgE antibodies can result. These antibodies have the ability to bind to the surfaces of mast cells and basophils; the subsequent interaction or exposure to allergen can cross-link two or more cell-membrane-bound IgE antibodies, leading to release of preformed mediators as well as synthesis and release of newly formed mediators [1]. Food-induced allergic reactions are responsible for a variety of symptoms involving the skin, gastrointestinal tract, and respiratory tract and might be a caused of life-threatening anaphylaxis. It is estimated that about 3-4% of adults and about 6% of young children are affected by food allergies and the prevalence is still rising [2-3]. Two invertebrate groups, crustacean (includes shrimp, crabs, lobster, and crawfish) and mollusca (includes snails, mussels,

oysters, scallops, clams, squid and octopus), are generally termed as “shellfish”. There are common constituents in the diet of many populations. Shellfish is one of the most common food allergens.

Tropomyosin is a heat stable major allergen in shellfish. It is a myofibrillar protein composed of two identical subunits with molecular mass of 35-38 kDa [4-5]. These molecules appear to be highly conserved in amino acid sequence. Tropomyosin is regarded as a pan-allergen that is implicated in IgE cross-reactivity among various invertebrates including crustaceans (decapod), mollusks, mites and cockroaches [6-8]. Tropomyosin cDNA sequences from black tiger prawn (*Penaeus monodon*), kuruma prawn (*Penaeus japonicus*), pink shrimp (*Pandalus eous*), king crab (*Paralithodes camtschaticus*), snow crab (*Chionoecetes opilio*), and horsehair crab (*Erimacrus isenbekii*) were elucidated by a cDNA cloning technique [9]. However, sequence of the tropomyosin from the freshwater prawn, *Macrobrachium rosenbergii* is not yet available. We report here the successfully of PCR amplification of a tropomyosin cDNA fragment from *M. rosenbergii*

Keywods:

tropomyosin, allergen, freshwater prawn, *Macrobrachium rosenbergii*

Materials and Methods

Freshwater prawn sample

M. rosenbergii (freshwater prawn) were purchased from Banglane, Nakhon-Pathom province. The muscle was dissected out and immediately frozen in liquid nitrogen and then stored at -80°C for molecular analysis.

Total RNA extraction

Total RNA was prepared from the muscle using Total RNA Extraction mini kit for Tissue (RBC) in order to use as template for amplified tropomyosin fragment.

Primers design

Primers were designed based on conserved amino acid sequences and nucleotide sequences of the tropomyosin from other shrimp species using Clustal W computer program (Fig. 1 and Fig. 2, respectively).

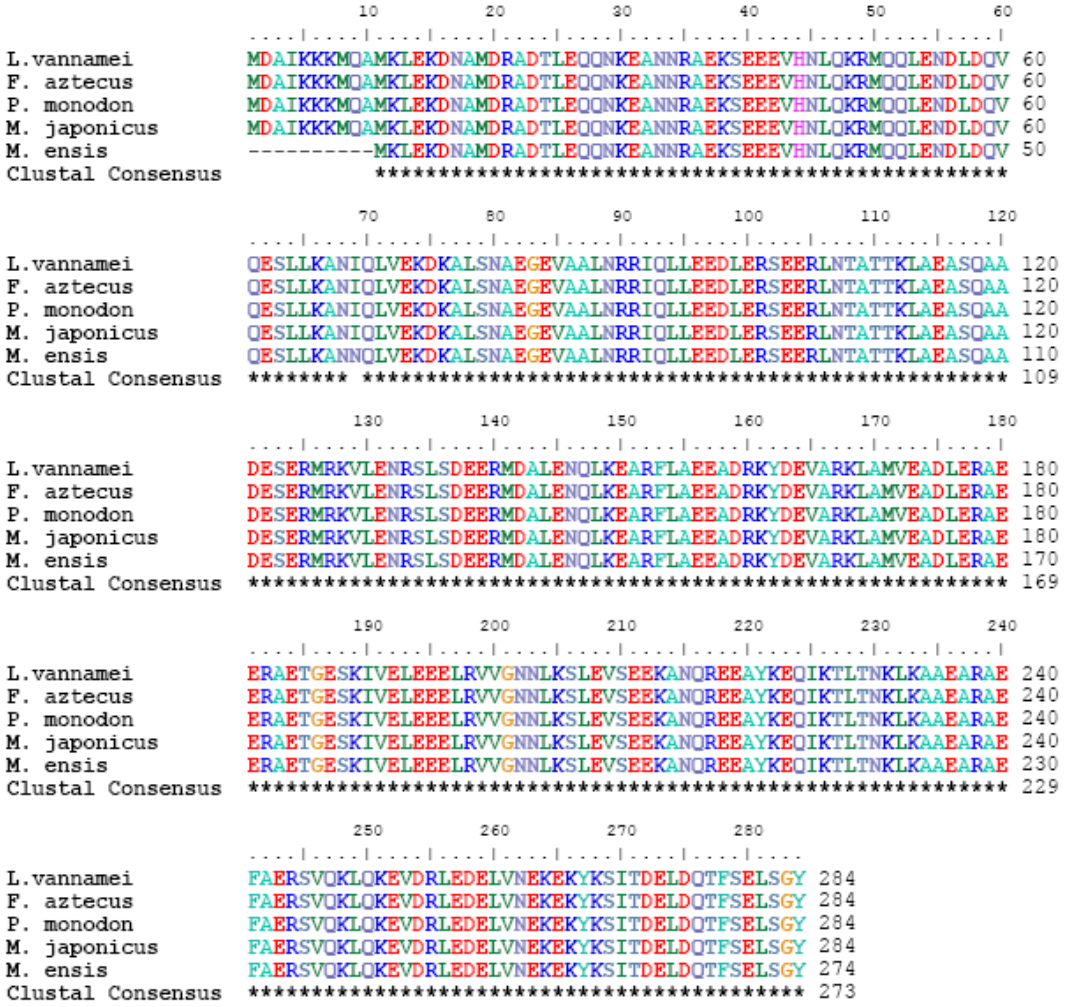

Fig. 1. Amino acid sequence alignment of shrimp tropomyosins. The shrimp names and their corresponding GenBank accession numbers are as following: *Litopenaeus vannamei* (white shrimp), ACB38288; *Farfantepenaeus aztecus* (brown shrimp), AAZ76743; *Penaeus monodon* (Black tiger shrimp), BAF47262; *Marsupenaeus japonicus* (Kuruma shrimp), BAF47263; *Metapenaeus ensis* (Greasyback shrimp or sand shrimp), Q25456. The numbers above the amino acid sequences indicate the position in the alignment of the amino acid residue. The (-) indicates a gap introduced into the amino acid sequence to allow for the maximum degree of identity in the alignment. Identical amino acid residue positions are indicated by asterisks.

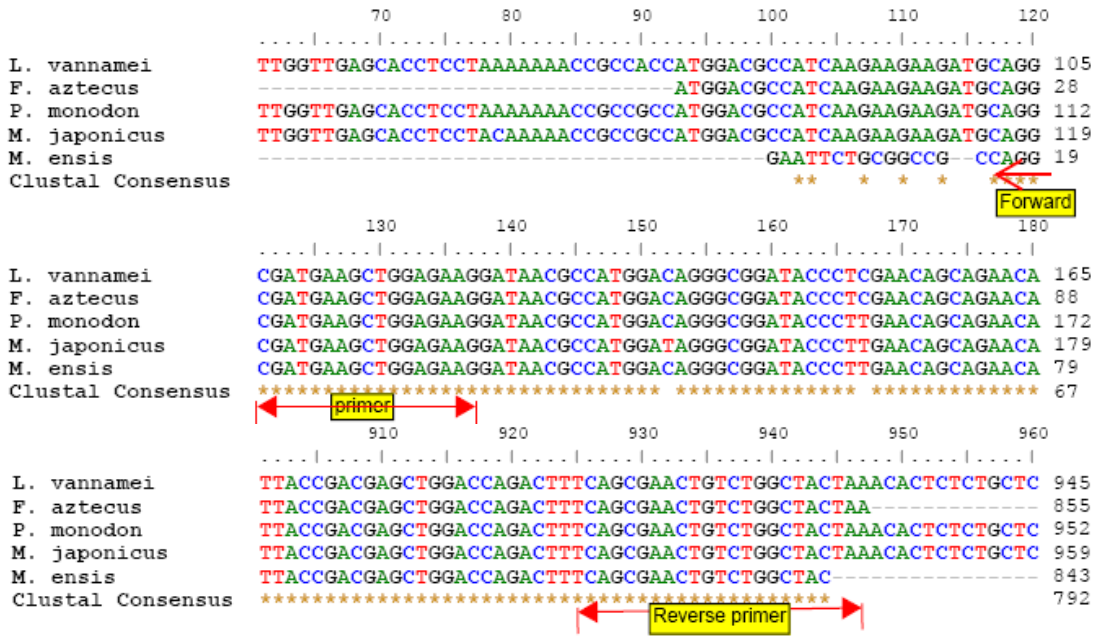

Fig. 2. Nucleotide sequence alignment of shrimp tropomyosins. The shrimp names and their corresponding GenBank accession numbers are as following: *Litopenaeus vannamei* (white shrimp), EU410072; *Farfantepenaeus aztecus* (brown shrimp), DQ151457; *Penaeus monodon* (Black tiger shrimp), AB270629; *Marsupenaeus japonicus* (Kuruma shrimp), AB270630; *Metapenaeus ensis* (Greasyback shrimp or sand shrimp), U08008. The numbers above the nucleotide sequences indicate the position in the alignment of the nucleotide residue. The (-) indicates a gap introduced into the nucleotide sequence to allow for the maximum degree of identity in the alignment. Identical nucleotide residue positions are indicated by asterisks.

Synthesis and amplification of cDNA fragment by One-Step RT-PCR

Reverse transcription (RT) and Polymerase Chain Reaction (PCR) was performed using the Titan One Tube RT-PCR System (Roche). Amplifications were carried out by using the pairs of specific primers. The PCR conditions were as follows: reverse transcription at 50^{o}C for 30 min, initial PCR activation step at 94^{o}C for 2 min, followed by 35 cycles of 94^{o}C for 30 sec, 50^{o}C for 30 sec and 68^{o}C for 45 sec, and then a final extension step was performed at 68^{o}C for 7 min. Each PCR reaction was conducted in a Mastercycler (GENE AMP PCR SYSTEM 2400, perkin elmer), in a 50 μl reaction mixture using 1 μg of total RNA, 200 μM of each dNTP, RT-PCR buffer (Tris-HCl, DMSO, 1.5 mM $MgCl_2$ pH 7.5), 10 mM forward (5'-CAGGCGATGAAGCTGGAGAAG-3') and reverse (5'-TTAGTAGCCAGACAGTTCGCTGA-3') primers, and 1 μl of Titan One-Tube RT-PCR enzyme mix (including AMV reverse transcriptase and Expand High Fidelity enzyme). The PCR products was kept at -20^{o}C, or immediately used for electrophoresis.

Results

The DNA fragment of tropomyosin gene of about 900 bp in length was amplified by PCR from freshwater prawn (Fig. 3). This fragment will be cloned and sequenced for investigate the open reading frame (ORF) of tropomyosin from freshwater prawn.

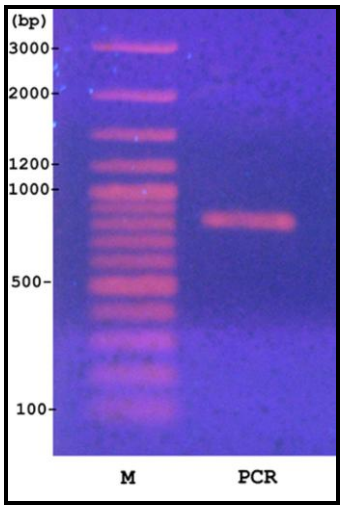

Fig. 3. PCR amplification of tropomyosin gene from freshwater prawn. M, DNA marker; lane 1, PCR amplification products.

Conclusion and Discussion

The present work reports a successful PCR amplification of a tropomyosin cDNA fragment of 900 bp which will be sequenced in the future. Open reading frame (ORF) of tropomyosin from *M. rosenbergii* was obtained by RT-PCR. 5' and 3' rapid amplification of cDNA ends (RACE) will be investigate in the future for understanding their evolution.

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Keywords; tropomyosin, freshwater prawn, allergen

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