

Identification and characterization of new allergen troponin C (Pen m 6.0101) from Indian black tiger shrimp *Penaeus monodon*

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Abstract Black tiger shrimp *Penaeus monodon* is the most consumed species of crustaceans all over the world. Seafood allergy is the major threat in developing and westernized countries. The use of recombinant allergen is an effective way to improve diagnosis and treatment for seafood allergy. In this study, the recombinant *P. monodon* troponin C (rPmTnC) was assessed for its sera collected from allergic individuals. The immunoblot results showed that the rPmTnC recognized by eight allergic individuals out of 35 confirmed the allergenicity of rPmTnC. This novel allergen could be used in allergy diagnosis and treatments. We have identified and cloned a new shrimp allergen Pen m 6.0101 from *P. monodon*.

Keywords *Penaeus monodon* · Troponin C · Allergen · Recombinant · Immunoblot · rPmTnC

Introduction

Seafood is a major source of food for human populations due to its dietary values [1]. Mainly, crustaceans are widely

consumed as delicious foods throughout the world [2]. Among the crustaceans, black tiger shrimp *Penaeus monodon* is a widely cultured species around the world. Though shellfish allergy is a long-lasting disorder typically persisting throughout life, shrimp cause most of the allergic food reactions [3]. In spite of the high prevalence of crustacean allergy, there is limited information regarding these allergens and its mechanisms involved in the induction of such allergic reactions. Seafood consumption rate plays a major role in the prevalence of seafood allergy [4]. Occupational allergy to shellfish has been reported in snow crab processing worker, shell grinders, cooks as well as restaurant workers [5, 6]. Development of new strategies in food processing and introduction of genetically modified foods are changing the nature of our diet [7] which also plays a role in food allergy scenario.

It has been reported that the range of *P. monodon* proteins (8–94 kDa) was reacted with serum IgE of allergic patients, but only few of them were identified and characterized as seafood allergens [8]. Muscle protein tropomyosin (36 kDa) was first identified as an allergen in shrimp, and it has been demonstrated as a pan-allergen in various seafoods, including shrimp [9–12], lobster [13], crab [14], squid [15], oyster [16], and octopus [17]. A minor shrimp allergen, arginine kinase (Pen m 2 and Lit v 2) has been identified in *P. monodon* and *L. vannamei*, respectively [8, 18]. Recently, sarcoplasmic calcium-binding protein has been identified as new allergen in *P. monodon* and *L. vannamei* (Lit v 4) [19, 20]. Myosin light chain, another muscle contraction protein, also has been identified as a new allergen in *L. vannamei* [21].

Troponin C (TnC) is a subunit of troponin which involves in calcium-dependent muscle contraction in skeletal and cardiac muscle. TnC is a member of a large group of Ca²⁺-binding proteins (CBPs) that also includes

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calmodulin, calpain, parvalbumin, calbindin, and myosin light chain [22]. TnC has been identified as an allergen in *Blattella germanica* (Blattella) with a calcium-dependent IgE reactivity [23]. Recently, the storage mite, *Tyrophagus putrescentiae* TnC, reported as a new mite allergen with calcium-dependent IgE reactivity [24].

Various methods for in vivo and in vitro in allergy diagnosis solely depend on the use of natural allergen extracts. Even though use of native allergens has proven to be more effective, the large-scale production and purity are found to be an obstruction for developing it as an effective allergen for diagnostic and treatment purposes. Additionally, this natural allergen extract contains non-specific allergenic components that has led to the need to use alternative strategies in allergy diagnosis and immunotherapy. It has been demonstrated that allergen extracts can be contaminated with allergens from other sources that cause false-positive [25]. Recombinant DNA technology allows production of large amounts of highly pure and well-defined allergens.

In this study, we report here the identification, cloning, and expression of a new allergen TnC of *P. monodon*. Efficacy of recombinant *P. monodon* troponin C (PmTnC) was assessed with sera of 35 allergic individuals. The results can provide the essential information for the use of TnC in seafood allergy diagnosis and immunotherapy.

Method

Sera from patients allergic to shellfish

Sera were collected from 35 volunteers with history of immediate allergic reactions after ingestion of shrimps and from three non-allergic subjects. A questionnaire was given to these volunteers to document history of type I hypersensitivity upon ingestion of shrimp. Sera from five subjects without any previous history of seafood allergy were used as controls. The study was approved by the institutional ethical committee.

Screening of shrimp *P. monodon* troponin C

Total RNA was isolated from shrimp *P. monodon* muscle tissue using Trizol reagent (Gibco BRL, USA). cDNA library was constructed using cDNA cloning kit according to the manufacturer's instruction (Invitrogen, USA). *P. monodon* cDNA library was screened for TnC genes using SP6 and T7 promoter primers by colony PCR. Sequence identity was confirmed using the BLASTX program. Based on the partial sequence information of MLC, full-length cDNA was synthesized using the RNA ligase mediated-RACE cDNA amplification kit according

to the manufacturer's instruction (Invitrogen, USA) with slight modifications. First-strand cDNA was synthesized using 1 µg of total RNA isolated as described previously. A 50-µl PCR mixture composed of 1 µl cDNA, 3 µl of Gene Racer 5' primer, 1 µl of gene-specific reverse primer (5'-TGCACGTCGATCTTGCCTGCG-3'), and 45 µl of Master Mix. Amplification was performed by 25 cycles involving 94 °C for 5 s, 65 °C for 10 s, and 72 °C for 3 min using the iCycler (BioRad, USA). The PCR product was resolved in 1.5 % agarose gel and gel-purified; the purified PCR product was cloned into pTZ57R/T vector (Fermentas, USA). Clones were sequenced and results were analyzed using the BLASTX program.

Multiple alignment results

Sequence similarity of PmTnC was done by BLAST analysis in nucleotide and amino acid level. The predicted amino acid sequence of PmTnC obtained in this study was aligned with other sequences of TnC from GenBank using CLUSTAL W (<http://www.ebi.ac.uk/ClustalW/>). The alignment results were used for further analysis with SignalP (<http://www.cbs.dtu.dk/services/SignalP>) to predict signal peptides of PmTnC protein. The functional motif and isoelectric point (pI) of PmTnC were predicted using MOTIF FINDER and Compute pI/Mw online tools (http://web.expasy.org/compute_pi/).

Allergen sequence homology comparisons

Sequence similarity search of PmTnC was performed with allergenic sequences available in SDAP database. SDAP allergenicity test was designed based on the FAO/WHO allergenicity rules (<http://fermi.utmb.edu/sdap/>) [26]. Single window 80 amino acids sequence similarity search of PmTnC was performed with allergenic sequences available in SDAP database.

Homology based model of PmTnC

The sequence of shrimp PmTnC was used for homology modeling. Templates were manually selected from the 46 TnC structures available in the Research Collaboratory for Structural Bioinformatics Protein Data Bank (<http://www.rcsb.org/pdb/>), on the basis of the highest degree of homology to the specific Pen m 6 (33 %). The sequence of TnC protein was aligned with that of the TnC of chicken *Gallus gallus* using ClustalW2. A 3D model of PmTnC protein has been built using the crystal structure of Incx molecule from *G. gallus*. Program Modeler was used for homology modeling. The stereochemical quality of the model was checked using the program PROCHECK and the Ramachandran plot was drawn. The model quality was assessed by

What-Check (through Swiss-Model and also available at <http://swift.cmbi.ru.nl/gv/whatcheck/>).

Cloning of PmTnC

A set of primers was designed for PmTnC open reading frame (ORF) incorporating the *Bam*HI and *Hind*III sites as restriction enzyme recognition sites (PmTnC F 5'-CGC GGA TCC ATG GAC TCC CTT GAT GAA GAG-3') and (PmTnC R 5'-CCC AAG CTT TTA ACC GCT CAT CAT CTC CAT-3'), respectively. Amplification was performed for 30 cycles involving 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min using the iCycler (BioRad, USA). The PCR product was resolved in 1.5 % agarose gel and gel-purified; the purified PCR product was digested with *Eco*RI and *Hind*III and cloned into pET32a vector (Novagen, USA). Clones were screened by colony PCR using T7 promoter primers and sequenced. Plasmid DNA was extracted from the cells using the DNA Purification Kit (Promega, USA) as per the manufacturer's instructions. Sub-cloning was performed using the recombinant plasmid containing *P. monodon* Troponin C (rPmTnC) with *E. coli* BL-21 (DE3) cells.

Expression of PmTnC

PmTnC was expressed as a fusion protein with thioredoxin (TRX) in *E. coli* BL21 (DE3) cells using 1 mM Isopropyl β -D-1-thiogalactopyranoside (IPTG) for 3 h at 37 °C. Bacterial lysate containing the rPmTnC was purified by denaturing method with 8 M Urea. IPTG induced bacterial cell pellet was resuspended in buffer A (8 M Urea, 25 mM Na₂HPO₄, 1 mM Tris-HCl, pH. 7.5), and the protein was purified from this lysate using Ni-NTA affinity column. The affinity column was washed with 20 volumes of buffer B (8 M Urea, 25 mM Na₂HPO₄, 1 mM Tris-HCl, pH. 6.5). The recombinant protein was eluted with buffer C containing 600 mM imidazole. Eluted recombinant PmTnC was dialyzed against 1X PBS with urea gradient (4, 2, 1, and 0 Urea), and the protein concentration was estimated by BCA protein assay kit (Pierce, USA). Purified rPmTnC protein content and purity were analyzed by SDS-PAGE.

Immunoblot

The allergenicity of the rPmTnC was determined in vitro by immunoblot assay using sera from 35 seafood allergic individuals. Briefly, 2 μ g of rPmTnC protein was resolved on a 12 % SDS-PAGE gel under reducing conditions and electrophoretically transferred onto PVDF membrane (BioRad, USA). Then, 2 μ g of purified TRX was used as negative control. Membrane was then blocked with 5 % nonfat milk powder in 1X PBST (PBS with 0.05 % Tween 20) at

4 °C for overnight. Allergic individual's sera were diluted (1:100) and incubated with the blots for 1 h at 37 °C. The membranes were then incubated with HRP-conjugated secondary IgE (1:5,000) (Sigma, USA) for 1 h at 37 °C. The signal was detected by incubating the membranes in DAB (3, 3'-Diaminobenzidine) for 5–10 min.

Results

Full-length cDNA of PmTnC

Screening and nucleotide sequencing of the *P. monodon* cDNA library containing PmTnC genes with different degree of truncations at the 5' end. Sequence alignment revealed that the three clones exhibited significant homology to TnC of *Homarus americanus*. Out of the three clones, clone 65 exhibited the longest size comprising of 849 bp with a polyadenylation sequence. Several rounds of screening the cDNA library failed to isolate the full-length PmTnC gene. The full-length PmTnC gene was synthesized by RACE PCR using gene-specific primers designed based on the sequence of the truncated clone 65 and 5'-RACE product. The 5'-RACE product and the 5'-truncated clone 65 exhibit a 343 bp overlap.

The full-length PmTnC is composed of 1,192 nucleotide bp with a 453 bp open reading frame from position 49 to 555 (Fig. 1). An ATG (M) initiation codon is found at position 87–89 while a TAA (stop) codon is found at position 537–539. The gene codes for the 150 amino acid protein with a predicted molecular weight of 16.8 kDa. No potential glycosylation sites were predicted in PmTnC. Sequence analysis revealed that PmTnC has high sequence identity with TnC isoforms 2a and 2b (81 %) from *H. americanus*, and it indicates that PmTnC is a member of the TnC isoform 2. Alignment results showed that PmTnC has 79, 61, and 59 % amino acid identity to Hom a 6, Bla g 6, and Per a 6, respectively (Fig. 2). In SDAP prediction, contiguous amino acid sequence similarity of PmTnC analyzed with SDAP allergen database shows that TnC has 50 % sequence identity with Hom a 6 from *H. americanus* (Fig. 3). Sequence analysis of this product provided the complete cDNA encoding for TnC from shrimp *P. monodon* (GenBank accession no. HM034316). PmTnC contains 2 EF-hand calcium-binding region (Fig. 4). An EF-hand motif consists of a 12 residue loop, flanked on both sites by α -helices.

Structural model of shrimp PmTnC

Several molecular models for PmTnC were constructed on the basis of other TnC structures. Figure 4 is a ribbon model view of the PmTnC, showing their structure

according to the sequence alignment given in Fig. 4a, b. Predicted two EF-hand calcium-binding regions of PmTnC shown in Fig. 4b. We used the program PROCHECK to validate the 3D structure of PmTnC. There is no deviation in the peptide torsion angle in the Ramachandran plot.

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1      acgggttttttttttttactggctcagcagcagtgagat      41
42    caccctagtgtaccgtgtcagctctcgccgcttaactcaagcaca      86
87    atggactcccttgatgaagagcagattgaaacctccgcaaggcc      131
      M D S L D E E Q I E T L R K R
132   ttcaactccttcgacactgaggcgccggctccatcaagcgcgag      176
      F N S F D T E G A G S I N A E
177   acggttgagtgatccttgaggatgatggcggtcaagatctctgag      221
      T V G V I L R M M G V K I S E
222   aagaactcgaggaggtcatcgccgagactgacgaggaaggctcc      266
      K N L Q E V I A E T D E D G S
267   ggtatgctgaggtcgaggagtttgctgagctcgccgccaagttc      311
      G M L E F E E F A E L A R K F
312   ctgatcgaggagcaggaagctctcaaggcgagctcagggaa      356
      L I E E D E E A R L K A E L R E
357   gccttcgtatctacgcaaggagctgcagggttacatcaccacc      401
      A F R I Y D K D C Q G Y I T T
402   gatattcctgaaggagatcttggtcgagcttgacccaagttagcc      446
      D I L K E I L V E L D P K L T
447   cccactgacttggaagcatttgaggaggtcgacgaggtggc      491
      P T D L E G I I E E V D E D G
491   tccggcactctgacttgatgaattcattgagatgatgagcggt      536
      S G T L D F D E F M E M S G
537   taaatcaccacttttcogatatccttcctcatctctcacctcttc      581
      T A A T C A C C A C T T T C O G A T A T C C T T C C T A T C T C T C A C C T C T T C
582   ttctattcattcctctatcctcacctctcagaaaaaaaatca      626
627   tctcaggtaaaaataaaacaaaagaagaaattagcgcattcatta      671
672   tggctcgtccgcaggtctctcatttttttctaaattatccttct      716
717   ccgtctctcttttgaattctctcgttctatgogccattcatctc      761
762   ttcaattcttaccactcaaccatttcagtcctgtgtatgatatt      806
807   ttacagtcattcattccttaagccttagggcaaaagtttttttt      851
852   ttttttttttatatgtccctcctccttcgactgaaataacaaca      896
897   acagcaaacaaacaaacaggaatatagacctcaattttcacct      941
942   ttatttttatgtaaagaagaggttcttctcctcctcgaggagaga      986
987   gagaggagatgatacatatcttataatttttagcagctatcgga      1031
1032  gttgcttatgatattcgttgagcattgacctgacagctgttgca      1076
1077  ttgcataaagtgggtgctgataacaaaaaacgacaaaaaaaac      1121
1122  tctcagggggcgagcattgaccttagagtcgataaaaatagctggc      1166
1167  gctgattttatcacgcgctgaaaaaa      1192

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Fig. 1 Nucleotide and deduced amino acid sequence of *P. monodon* troponin C. The deduced amino acid sequences are denoted below the nucleotide sequences. The predicted initiation (atg) and stop codon (taa) are *underlined*. The predicted N-glycosylated site (N-S-F-D, aa 17–20) is in *italics* and *underlined*

Fig. 2 Multiple alignment results of *P. monodon* troponin C. Comparison of the deduced amino acid sequences of *P. monodon* troponin C with other crustacean and arthropod allergen sequences, *H. americanus* (Hom a 6), *B. germanica* (Bla g 6), and *P. americana* (Per a 6). The numbers at the right margin indicate the position of the amino acids. The predicted EF-hand calcium-binding protein functional motifs are shown in box. Identical (asterisk), conserved (colon) and semi conserved (dot) residues are indicated

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Bla g 6  MDELPEQIQLKKAFDAFDREKKGCISTEMVGTILEMLGHLRDDMLQEIIAEVDADGS 60
Per a 6  MDELPEQIQLKKAFDAFDREKNGFISTDMVGTILEMLGHLPLDDMLEEIIAEVDADGS 60
Tro C   MDLDEEQLTLRKAFNSFDTEGAGSINAEIVGVILRMGVKISEKQLQEVIAETDEDGS 60
Hom a 6  MDSLDAEQLSALQKAFDSFDTSKGFITPETVGVILRMGVKISEKQLQEVISETDEDGS 60
      **,*  **,:  *:***:***  :  *  *..:  **,*,*:*  :..  *:***:  *  ***

Bla g 6  GELEFEFFVSLASRFVEEDAEMQELKEAFRLYDKEGNGYITTNVLRILKELDDKIT 120
Per a 6  GELEFEFFVTLAARFVEEDAEMQELKEAFRLYDKEGNGYITTTVLRILKELDDKIT 120
Tro C   GMLEFEFFRELARKFLIEDEEALKAELEAFRLYDKCQGYITTDILKEILVELDPKLT 120
Hom a 6  GELEFEFFVELARKFLIEDEEALKAELEAFRVYDRGGNGYITTDVLKEILVELDNRLT 120
      *  ***:*  **::**:*  ***:***:***:  :*****  *:***  ***  ::*

Bla g 6  AEDLDMMIEEDSDGSGTVDFDEFEVMTGE 151
Per a 6  NEDLDAMIEEDSDGSGTVDFDEFEVMTGE 151
Tro C   PTDLEGIIEEVEDGSGTLDFFDEFEVMTGE- 150
Hom a 6  EEDLDLIEEVEDGSGTLDFFDEFEVMTGE- 150
      **:  :***:  :*****:***:***:***:***:

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Expression and purification of rPmTnC

Complete ORF of PmTnC was successfully amplified and cloned into pET32a vector (Fig. 5a). rPmTnC expression was obtained in IPTG induced *E. coli* BL-21 (DE3) cells. SDS-PAGE results revealed the presence of a 36.5 kDa band representing rPmTnC (16.8 kDa) including the 19.7 kDa TRX fusion protein (Fig. 5b). The rPmTnC was expressed as inclusion bodies, and it was successfully purified under denaturing conditions. It was estimated that the purified rPmTnC protein with a yield of 18 mg protein from 100 ml culture of bacterial cells.

Serum IgE reactivity

Sera from 35 allergic individuals revealed the presence of sero-reactive bands seen at 16.8 kDa along with TRX fusion protein indicating the presence of rPmTnC protein. Results of immunoblot showed positive reaction with collected allergic individual's sera. Figure 6 summarizes the results of immunoblot with rPmTnC. No bands were observed in all the control sera of non-allergic individuals (C1–C5).

Discussion

Shrimp are the major cause of allergic reaction among the shellfish. To date, only few IgE-binding proteins from shrimp have been identified as allergens. Tropomyosin was first identified as a major allergen in shrimp and considered as a pan-allergen among the crustaceans, molluscs, and arthropods [9, 10, 27]. Addition to this, arginine kinase (Pen m 2 & Lit v 2), a minor allergen has been reported [8, 18]. Most recently, MLC (Lit v 3) and SCP (Lit v 4 & Pen m 4) also have been reported as shrimp allergens [20, 21].

Fig. 3 SDAP allergen prediction of PmTnC. Contiguous amino acid sequences were *highlighted*. The variations in the amino acid sequences are marked *without highlighting*

TnC	1	MDSLDEEQIETLRKAFNSFDTEGAGSINAETVGVILRMGVKISEKNLQE	50
Hom a 6	1	MDSLAEQLSALQKAFDSFDTDSKGFITPETVGVILRMGVKISEKNLQE	50
TnC	51	VIAETDEDCSGMLEFEFFAELAAKFLIEEDEEALKAELEAFRIYDKDCQ	100
Hom a 6	51	VISETDEDCSGMLEFEFFVLAALKFLIEEDEEALKAELEAFRVYDRGGN	100
TnC	101	GYITTDILKEILVELDPKLTPTDLEGIIEEVEDEGSGTLDLDFEFMEMMSG	150
Hom a 6	101	GYITTDVLKEILRELDNRLTEEDLDSIEEVEDEGSGTLDLDFNEFMQMMNG	150

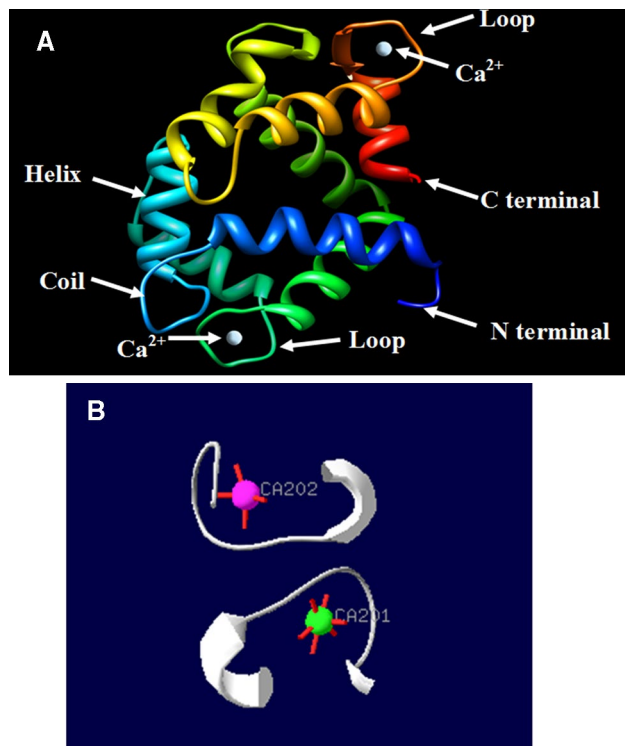


Fig. 4 Molecular model of TnC. **a** PmTnC 3D structure was built with modeler server. Arrows indicate the helix, coil, and loop. **b** Two EF-hand calcium-binding domains predicted as a functional motif in PmTnC is shown

In this report, PmTnC was identified as a new shrimp allergen, Pen m 6.0101. The obtained 5' UTR region of PmTnC mRNA sequence was short in length. 5' UTR is highly correlated with translational regulation, especially for the regulation on initiation of translation. Thus, the variations of the length of 5' UTR can significantly improve the regulation of gene expression, even though the rPmTnC protein expression level is high in bacterial system.

Nucleotide analysis revealed that PmTnC gene is 453 bp in size encoding 150 amino acid residues and an estimated molecular weight of 16.8 kDa with calculated *pI* of 3.91. Deduced nucleotide and amino acid results revealed high similarity to Hom a 6, Bla g 6, and Per a 6 of *H. americanus*, *B. germanica*, and *P. americana*, respectively. Shrimp PmTnC is

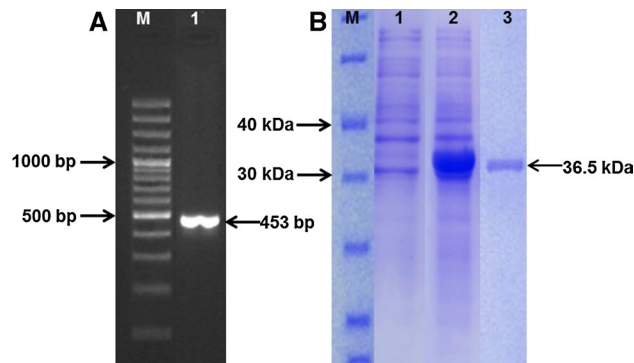


Fig. 5 Amplification of PmTnC. **a** Amplification of complete ORF's of TnC gene from *P. monodon* by PCR using gene-specific primers. Lane M DNA ladder (100 bp–4 kb), Lane 1 TnC (453 bp). **b** Expression of rPmTnC induced by IPTG with a final concentration of 1 mM. rPmTnC protein was expressed along with thioredoxin (TRX) fusion protein (19.7 kDa). Lane M protein marker (low-range marker), Lane 1 *E. coli* BL21 (DE3) cells (uninduced), Lane 2 TnC (36.5 kDa), and Lane 3 purified rPmTnC

the EF-hand type calcium-binding protein and contains two Ca^{2+} ions in each chain. These results indicate that the shrimp PmTnC is a member of TnC isoform 2 and could be a cross-reactive allergen with other invertebrates at low level.

PmTnC was combined with the expression vector pET32a and expressed in *E. coli* BL-21 (DE3). The fusion protein was insoluble and exhibited about 36 kDa in size on SDS-PAGE, identical to the predicted molecular weight of PmTnC. The expressed rPmTnC fusion protein was recognized by IgE sera from allergic subjects by immunoblotting, indicating that the recombinant protein has allergenicity with IgE-binding epitopes.

In immunoblot, rPmTnC reacted with patients in low level and suggested that the wild-type recombinant proteins may not be able to elicit strong IgE as that of the native allergens. Lack of post translational modification in *E. coli* may be the possible reason for the reduced activity. rPmTnC reacted to sera collected from occupational workers such as seafood processing workers and restaurant workers and fisher women's. Previously, it has been demonstrated that recombinant MLC (Lit v 3) is also a member of calcium-binding proteins that binds to allergic patients at low levels compared to the native allergens [21].



Fig. 6 Sero-reactivity of rPmTnC. Immunoblot analysis of rPmTnC using 35 allergic patient's sera. Lane M protein molecular weight marker. Lane 1–35 bands showing the binding of IgE with TnC (Lane

1–3, 7, 9, 11, 18 and 24 showing positive reaction to allergic patient's sera). Arrow indicates the binding of IgE with rPmTnC. Lane C1–C5 no reaction with control sera

PmTnC is EF-hand type CBPs. The canonical EF-hand is a highly conserved portion of the protein and consists of an alpha helix, a loop around the bound calcium ion, and a second alpha helix. PmTnC showed the calcium placed in the helix-loop-helix conserved portion of calcium-binding proteins like other predicted 3D structures. It is shown that calcium bound form of EF-hands shows high IgE-binding activity than the calcium depleted form suggesting a role for this EF-hands in allergenicity [28]. Calcium-dependent modulation of IgE binding has been demonstrated with a variable number of EF-hand motifs in tree pollens (Bet v 4, Ole e 3, Ole e 8), grass pollens (Phl p 7), rapeseed (Bra n 1, Bra n 2), and some vertebrates such as fish (Gad m 1, Sal s 1) and frog (Ran e 1, Ran e 2), [29, 30]. Calcium-dependent IgE binding has been reported for several calcium-binding allergens. IgE binding was increased by addition of calcium to sera previously calcium depleted with EGTA. Inhibition studies among different EF-hand proteins from pollen and vertebrates showed limited cross-reactivity [31]. This possibly suggests that different families of calcium-binding allergens may possess specific epitopes involved in cross-reactivity within members of the same family. This has been reported the calcium-binding allergens are highly cross reactive [28]. All these results confirmed shrimp PmTnC member of calcium-binding protein as a new allergen from *P. monodon*.

In summary, we have identified shrimp TnC as a new shrimp allergen named Pen m 6.0101. Even though Pen m 6.0101 appears to be a minor allergen, sequence results indicate that they might be a cross-reactive allergen with other invertebrates at low level. Results of recombinant allergen elucidates that these can be used in allergic diagnosis and allergen immunotherapy. Further, this will also help in developing new and effective strategies for diagnosis and treatment of seafood allergy.

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Conflict of interest None.

Compliance with Ethics Requirements All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). Informed consent was obtained from all patients for being included in the study.

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