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SHORT COMMUNICATION

Isolation and Cloning of Tropomyosin and Arginine Kinase from Tiger Prawn *Penaeus monodon* and Blue Swimming Crab *Portunus trituberculatus*

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INTRODUCTION

Shellfish is an important source of food and plays a significant role in human nutrition and health. However, shellfish allergy is a long-lasting disorder which mostly persists throughout life and is often associated with severe reactions (Fu et al., 2019). Among various commercial shellfish available, prawns and crabs are the most widely consumed and can lead to the most severe allergenic reactions. At present, allergies to shellfish are diagnosed similarly to other food allergies. The diagnosis relies upon careful evaluation of patients' history, presence of appropriate clinical signs and confirmation with *in vivo* skin prick testing or *in vitro* measurement of specific immunoglobulin E (IgE) tests to demonstrate the presence of allergen-specific IgE (Roberts et al., 2016). However, both *in vivo* or *in vitro* diagnostic approaches are mainly based on the use of crude allergen extracts.

Crude allergen extracts are obtained from biological sources and consist of a mixture of allergenic components with high amounts of undesirable products that can interfere with the diagnosis because the tests are not capable of differentiating between primary sensitization and immunological cross-reactivity. This causes difficulties for clinicians in their day-to-day work of interpreting the results of the allergy tests. In many cases, only a few of the several proteins found in crude allergen extracts act as the essential allergens towards patients who are allergic to the substance. The most important ones are called major allergens.

Problems associated with using crude allergen extracts for allergy diagnosis can be overcome with the usage of recombinant allergens. Recombinant allergens with high purity can be produced by using controlled production methods that yield defined molecules with known molecular, immunologic, and biological characteristics (Fu et al., 2019). Tiger prawn, *Penaeus monodon* and blue swimming crab, *Portunus trituberculatus*, are among the widely consumed shellfish in Malaysia. Our earlier study involving 131 atopic patients in Allergy Clinic, Kuala Lumpur Hospital demonstrated that patients in Malaysia suffering from allergic responses to shellfish that includes tiger prawn, *Penaeus monodon,* and blue swimming crab, *Portunus trituberculatus*. Amongst the shellfish tested using crude extracts, prawn elicited the highest

frequency of positive reactivity in 39% of the patients followed by crab, which elicits a positive reaction in 24% of the patients (Zailatul, 2017).

RESULTS AND DISCUSSION

In the first phase of our study, we successfully identified tropomyosin and arginine kinase as the major allergens in both species of shellfish. However, more information about the individual allergenic species-specific components is needed. Therefore, we continued our study to isolate and clone the tropomyosin and arginine kinase from these two species of shellfish, tiger prawn *Penaeus monodon,* and blue swimming crab *Portunus trituberculatus*. Tropomyosin and arginine kinase were isolated from the total RNA (Ribonucleic Acid) obtained from both prawn and crab muscles followed by RT-PCR (Reverse Transcriptase-Polymerase Chain Reaction). The RT-PCR products were then cloned into the cloning vector, pJET 1.2 and transformed into *Escherichia coli* host. Transformants were screened for positive clones by PCR (Polymerase Chain Reaction) colony and sequenced. The 855 bp tropomyosins have been isolated and sequenced from both prawn and crab.

Arginine kinases isolated and sequenced from prawn and crab were 1071 bp and 1074 bp, respectively (Figure 1). The GenBank BLAST search for the sequences showed high homology to the targeted proteins as shown in Table 1. Tropomyosin is a 34 to 38 kDa heat-stable protein that belongs to a highly conserved family of actin filament binding proteins, which plays a functional role in contractile activities in muscle cells (Ruethers et al., 2018). Arginine kinase is a 40 to 42 kDa heat-labile protein that plays an important role in regenerating adenosine triphosphate (ATP) during bursts of cellular activity (Yang et al., 2019). Tropomyosin and arginine kinase from the prawn and crab have been isolated and the full-length sequences were obtained.

Thus far, our study is the first in Malaysia to report successful isolation and cloning of tropomyosin and arginine kinase from tiger prawn, *Penaeus monodon* and blue swimming crab, *Portunus trituberculatus*. Current ongoing study focuses on sub-cloning and full-length expression of tropomyosin and arginine kinase in order to produce respective recombinant proteins, and subsequently investigate their physicochemical and allergenic characteristics. This study can serve as a prototype study in Malaysia to express other identified allergen molecules such as myosin light chain, sarcoplasmic calcium binding protein, troponin C, triose-phosphate isomerase and hemocyanin to complete the allergen panel in prawn and crab.

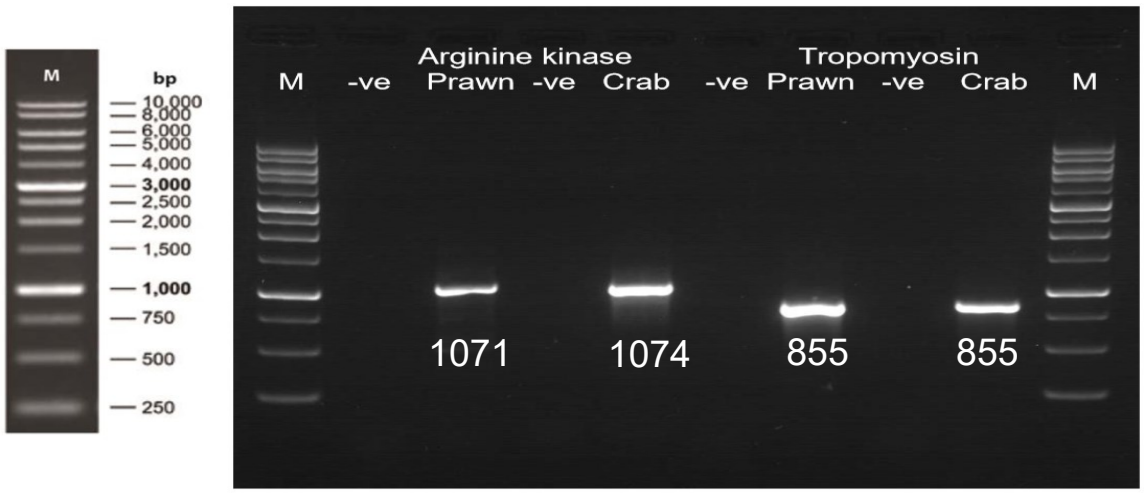

Figure 1 Detection of PCR products.

Table 1 Sequences analysis.

No.	Protein	Accession number	Blast score	Nucleotide identities (%)
1	*Portunus trituberculatus* arginine kinase	HQ214139.2	1902	99%
2	*Portunus trituberculatus* tropomyosin	EF672352.1	1557	99%
3	*Penaeus monodon* arginine kinase	GQ246164.1	1973	99%
4	*Penaeus monodon* tropomyosin	AY827100.1	1574	99%

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