Characterization studies of gill-specific crustin isoform (MjCRS) and WAP four-disulfide core domain-like gene (MjWFDC-like) in kuruma shrimp Marsupenaeus japonicus

［課程博士・論文博士共通］

博士学位論文内容要旨
Abstract

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論文題目 Title	Characterization studies of gill-specific crustin isoform (MjCRS) and WAP four-disulfide core domain-like gene (MjWFDC-like) in kuruma shrimp *Marsupenaeus japonicus* クルマエビ *Marsupenaeus japonicus* エラ特異的クラスチンおよび WAP four-disulfide core domain 様遺伝子の研究		

Shrimp aquaculture is one of the important industrial practices in the world. Much of the livelihoods are depend on this industry. However, this industry has been under constant threat of various bacterial and viral disease outbreak. Hence, shrimp diseases have become the major concern, not only because of its economic loss but also because of its impact on the livelihood that depend on shrimp aquaculture industry.

The white spot disease and the acute hepatopancreatic necrosis disease are two of the most serious diseases of shrimp industry. Many different strategies such as prevention, detection and treatment have been developed to find the solution against disease. However, detection and treatment are post disease occurrence strategies which are not use for complete cure of disease. Hence, as a part of preventive measure, understanding the shrimp immunity is very important that could help to create robust preventive measure against invading pathogens.

To understand shrimp immunity, many researchers have conducted immune related gene studies. These studies were conducted from different key organs of shrimp such as hemocytes, lymphoid organ, hepatopancreas, and gills. In our laboratory, previously conducted microarray analysis of immune related organ of kuruma shrimp *Marsupenaeus japonicus* showed many crustin peptide reads in gills tissue. These reads were further validated by RT-PCR analysis. Based on these previous results, the current study focuses on characterizing gill-specific crustin isoform from kuruma shrimp *M. japonicus*.

Crustins are cysteine-rich antimicrobial peptides (AMPs) that are recognized by the presence of a signal peptide at the amino terminus followed by a multidomain region (glycine-rich, proline-rich, or cysteine-rich) and a whey acidic protein domain (WAP) at the carboxyl terminus. They are considered as very enigmatic molecule due to its varied sequential and functional characteristics. There are mainly three type of crustins (type1-3) that are mainly categorized based on its differences in its multidomain region and number of WAP domain. Type 1 crustins are cysteine-rich region between a signal peptide and a WAP domain. Type 2 crustins are categorized based on its characteristics of glycine-rich region following signal peptide in addition to cysteine-rich region in the center and a WAP domain. Type 3 crustins contained either a short proline or arginine-rich region in-between a signal peptide and a WAP domain.

Type 2 crustins have many diversified isoforms and they are most abundant type of crustins that are common in shrimps. They play a critical role in shrimp humoral innate immune response that were proved from many previous studies that focused on its tissue distribution analysis following experimental infection by different pathogens. Majority of previous study characterized crustins isoform from hemocytes. However, there is a few information available on its presence in other tissues.

Previously generated expressed sequence tags (EST) from kuruma shrimp gills transcriptome showed high similarity with existing crustin in NCBI database with different species of shrimps, crabs and lobsters. These sequences similarities were ranged from 37-100% with crustins from *M. japonicus, Farfantepenaeus subtilis, Litopenaeus satiferus, L. vannamei, F. paulensis, Penaeus monodon, Macrobrachium rosenbergii,*

Panulirus japonicus, Portunus trituberculatus and *Scylla paramamosain*. In phylogenetic tee analysis, these sequences showed thirteen possible crustin variants. Further, tissue expression of these sequences revealed its presence in different tissues. Thus, from these results locus 3886(1), locus 16076, locus 11885, locus 2365 and locus 5809 were selected for further analysis. In multiple sequence alignment of these sequences with previously identified kuruma shrimp crustin isoform MjCRS1-5 and with highly similar crustin sequences from NCBI database showed high identity in its carboxyl terminus that contained WAP domain. ORF finder of locus 2365, locus 5809 and locus 3886(1) showed partial characteristics of crustin where locus 2365 and locus 5809 showed a cysteine-rich region and a WAP domain, and locus 3886(1) showed a glycine-rich region and a cysteine-rich region and a WAP domain. On the other hand, locus 16076 and locus 11885 showed a full length ORF that contained all the characteristics of crustin. Thus, primers were design from these potentials crustin peptide EST sequences to investigate its full length ORF sequences from kuruma shrimp gills cDNA.

The results showed full length ORF sequence of locus 3886(1), locus 16076, locus 2365, locus 5809. Hence, after confirming all the characteristic of crustin they were renamed as MjCRS6, MjCRS7, MjCRS8 and MjCRS9, respectively. An open reading frame of MjCRS6, MjCRS7, MjCRS8 and MjCRS9 were 513 bp, 489 bp, 593 bp and 459 bp encoding a deduce amino acid of 170 aa, 162 aa, 197 aa and 152 aa, respectively. MjCRS6, MjCRS7 and MjCRS9 transcript were only detected in gills tissue and not in other tested tissue. While, MjCRS8 transcripts were mainly expressed in gills and a weak band were observed in other tissue. The phylogenetic tree analysis classified MjCRS6-9 as type 2 crustin family isoform. To check MjCRS6-9 role in shrimp immunity, a time dependent gene expression studies were conducted twice, either against *V. parahaemolyticus, V. penaeicida* or WSSV. However, they showed no significant difference in gene expression at 3 h, 6 h, 12 h, 24 h and 48 h when data were compared with 0 h group.

Locus 11885 showed full length ORF that contained all the characteristic of crustin. However, bioinformatics analysis did not show a WAP domain with high confidence. The sequence analysis suggested it as an imitation of crustin sequence. Hence, it was designated as a new MjWFDC-like gene. Tissue distribution profile suggested that MjWFDC-like is gills-specific gene. MjWFDC-like multiple sequence alignment results reveled WFDC alike domain at the carboxyl terminus. In phylogenetic tree analysis with crustin sequences and WFDC domain sequences, MjWFDC-like created a separate clade. Considering the antimicrobial properties of WFDC domain, a challenge tests were conducted against *V. parahaemolyticus, V. penaeicida* or WSSV. However, MjWFDC-like gene expression was not significantly upregulated after infection.

Taken together, current study revealed four new crustin isoform MjCRS6-9 and a MjWFDC-like gene from kuruma shrimp *M. japonicus*. The multiple sequence alignment and phylogenetic tree analysis classified MjCRS6-9 as type 2 crustin family. The multiple sequence alignment of MjWFDC-like gene showed a variant of WFDC domain that was designated as WFDC alike. MjCRS6-9 and MjWFDC-like transcripts were mainly detected in gills tissue. The gene expression analysis of MjCRS6-9 and MjWFDC-like did not show any significant upregulation against *V. parahaemolyticus, V. penaeicida* or WSSV.

The results of this study provide the basis to hypothesis the different origin of crustin based on the its presence solely in gills. It might be possible that these gills-specific crustin isoform might have different function in shrimp. However, further study is required to investigate its functional characteristics. The presence of many WFDC domain sequences in GenBank indicated the existence of it in diverse forms of animal life. However, they are poorly understood. Thus, the newly discovered MjWFDC-like gene data would help to find their biological significance in different forms of animal life.