The immune defense of shrimp gills revealed by Marsupenaeus japonicus gill C-type lectin (MjGCTL)

［課程博士・論文博士共通］

Summary

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論文題目 Title	The immune defense of shrimp gills revealed by *Marsupenaeus japonicus* gill C-type lectin (MjGCTL) (クルマエビのエラ C 型レクチン研究によるエラの生体防御機構の解明)		

Gills of penaeid shrimp act as the gateway between internal and external environment, functioning in gas and ion exchange, filtering out harmful biotic and abiotic factors. To the immune system, gills are known to merely assist mechanically through the removal of trapped foreign materials during molting. Here, we demonstrate that shrimp gills are also equipped with other immune molecules present in gills, and such is the gill C-type lectin of Marsupenaeus japonicus (MjGCTL). The molecular characterization of MjGCTL was done using recombinant (r)MjGCTL protein produced from Drosophila S2 cells. This demonstrated MjGCTL's role as a C-type lectin functioning as a pathogen recognition protein (PRR) capable of binding to carbohydrates on the bacterial surface causing bacterial agglutination. As a PRR, MjGCTL can also be recognized by shrimp hemocyte cells to facilitate encapsulation and malanization of foreign agents. MjGCTL protein was detected on the gill surface mucus, which also displayed bacteria agglutinating activity. Evidence confirming that MjGCTL is involved in this agglutination activity of the gill mucus was provided by the inhibition of agglutination ability upon adding MjGCTL's antibody to the gill mucus neutralizing MjGCTL. Furthermore, the native MjGCTL purified from gill tissues displayed similar protein functions with rMjGCTL, where the eluted native MjGCTL displayed bacterial agglutinating activity. in vivo Phagocytosis assay by flow-cytometry using PKH67-labelled Streptococcus agalactiae revealed that the eluted native MjGCTL can also act as an opsonin, increasing the phagocytic rate. The function of MjGCTL in vivo was done through the knockdown of MjGCTL by RNAi, followed by challenge test by bacterial immersion using a low bacterial concentration of Vibrio parahaemolyticus. Results showed that the knockdown of MjGCTL made shrimp more vulnerable to infection reducing survival to 0% at 7-day post-infection as compared to the control groups that maintained 90% survival. This can be explained by the impaired ability of the gill mucus to agglutinate bacteria that is observed among MjGCTL-knocked down shrimp. Also, increase of total vibrio and bacteria was observed in both gill and hemolymph microflora among MjGCTL-knocked down shrimp. Upon bacterial immersion, the knockdown of MjGCTL caused a significant increase in bacterial load at 6 to 12-hour post-immersion was observed in both gills and hemolymph of MjGCTL-silenced shrimp. Since MjGCTL do not have a direct antimicrobial property, these changes in the bacterial load can be results of the downregulation of other antimicrobial peptides, specifically crustin and penaeidin, caused by knockdown of MjGCTL. Furthermore, a microarray analysis of other novel genes, revealed 27 immune-related genes upregulated (>2 fold) only in gill tissues with comparison to other immune-related organs. These results are evidences that shrimp gill provides not only a physical, but also a biochemical barrier lined with immune molecules such as MjGCTL.