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学 位 論 文 要 旨
Dissertation Abstract

氏名：
Name　Indri Afriani Yasin

学位論文題目：
Title of Dissertation　Effects of functional polysaccharides derived from insects on cultured prawns
（昆虫由来機能性多糖の養殖エビに対する効果に関する研究）

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Crustaceans are one of the most traded aquaculture products in terms of commercial value. Half of the total productions of crustaceans is dominated by *Litopenaeus vannamei* or commonly named as white shrimp. In aquaculture developments efforts, disease is one of the causes of production failure, including on shrimp farming. However, prawn production and price fluctuations are associated with the outbreak of microbial diseases. Disease such as vibriosis have been responsible for severe economic losses by causing mass mortalities for shrimp farming.

Since a long time ago, researchers and farmers are working hard to prevent disease. The popular solution that has been emerged is vaccination and the use of antibiotic. But, all of the data suggests that shrimp immune systems can not respond to vaccination, and antibiotic treatment can be harmful to the natural environmental and can stimulates the mutation of antibiotic-resistant strain of bacteria or viruses.

Insects is one of the promising solution for a new, safe, and effective techniques to deal against diseases in aquaculture activity. In this study, the author focused on character and biological activity of silkrose in prawn, and reported establishment of challenge study system for the vibriosis of Japanese tiger prawn with a high virulent *Vibrio Penaeicida* strain to develop a model system for bacterial disease in crustacean species.

In chapter 1, to demonstrate efficacy of Silkrose® for infectious disease of Japanese tiger prawn, the author performed challenge study with a high virulent *Vibrio penaecidae* strain. To confirm effective infectious route and dose, we performed two-injection methods with several dilutions. As the results, all

prawns turned to be dead within 1-3 days in dose dependent manner in intramuscular injection and within 7 days in immersion groups. Underlying cellular and molecular mechanisms of immunization in crustacean remain elusive, but Silkrose® is expected to serve as a new tool to control diseases of crustacean species in aquaculture production.

In chapter II, previously the author identified novel bioactive polysaccharides from *Bactrocera cucurbitae* and *Antheraea yamamai* that activate innate immunity in RAW264 murine macrophages. However, in terms of potential applications in the cultivation of prawns, there were problems with the availability of these insects. However, the author identified a polysaccharide from *Bombyx mori* that activates innate immunity in RAW264 cells and penaeid prawns. This purified polysaccharide, termed silkrose of *B. mori* (silkrose-BM), has a molecular weight of 1,150,000 and produces a single symmetrical peak on HPLC. Eight of nine constitutive monosaccharides of silkrose-BM are concomitant with dipterose of *B. cucurbitae* (dipterose-BC) and silkrose of *A. yamamai* (silkrose-AY). The major differences are found in the molar ratios of the monosaccharides. Silkrose-BM is approximately 500-fold less potent than silkrose-AY (EC50: 2.5 and 0.0043 μg/mL, respectively) in a nitrite oxide (NO) production assay using RAW264 cells. However, the maximum NO production for silkrose-BM and AY were comparable and higher than that of the lipopolysaccharide of *Escherichia coli*.

The survival of penaeid prawns (*Litopenaeus vannamei* and *Marsupenaeus japonicus*) after infection with *Vibrio penaecida* was significantly improved by both dietary silkrose-BM and *B. mori* pupae. This suggests that silkrose-BM effectively prevents vibriosis in penaeid prawns via the activation of innate immunity.

In this chapter also the author used the Hepatopancreas to monitor the effect of various dosage of silkrose. In this study histological alterations were observed in Hepatopancreas of *L.vannamei* with diets containing silkrose of B.mori. The result show the number of Bcells and Rcells increased throughout the tubule in shrimp fed with silkrose 0.25 μg/g. In conclusion, bioactive substances like silkrose will become more important tools for bacterial disease protection in future prawn cultivation