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学 位 論 文 要 旨
Dissertation Abstract

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学位論文題目： Insect-derived functional polysaccharides: A potential alternative for disease control in fish and shellfish
Title of Dissertation (昆虫由来機能性多糖：魚介類疾病防除物質としての可能性)

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This dissertation reveals the presence of novel insect-derived polysaccharides from silkmoth (*Bombyx mori*), Japanese oak silkmoth (*Antheraea yamamai*), and black soldier fly (*Hermetia illucens*) and demonstrates their effects as an immunomodulatory substance by *in vitro* and *in vivo* analysis. In this study, three novel insect-derived polysaccharides were identified in the *B. mori* pupae, *A. yamamai* pupae, and *H. illucens* larvae, named silkrose-BM, silkrose-AY, and dipterose-BSF, respectively. These bioactive substances can induce the activation of innate immune responses in mammalian macrophages. To isolate these molecules, a series of purification steps, including water extraction, ethanol precipitation, gel-filtration, and anion-exchange chromatography, was conducted followed by nitric oxide (NO) production assay in mouse RAW264.7 macrophage cell line as a marker of immunomodulatory activity. High-performance liquid chromatography showed that the average molecular weight of silkrose-BM, silkrose-AY, and dipterose-BM was estimated to be 1.15×10^6 da, 3.15×10^5 da, and 1.47×10^5 da, respectively. Gas chromatography-mass spectrometry utilizing acid hydrolysis and acetylation techniques revealed that silkrose-BM, silkrose-AY, and dipterose-BSF consisted of nine, nine, and ten monosaccharides, respectively. Furthermore, *in vitro* analysis demonstrated that silkrose-BM, silkrose-AY, and dipterose-BSF enhanced the expression of proinflammatory cytokines in RAW264.7 cells. The suppression of the activities of Toll-like receptor 2 (TLR2) and/or 4 (TLR4) significantly attenuated NO production by silkrose-BM, silkrose-AY, and dipterose-BSF, suggesting that these purified polysaccharides stimulate the secretion of various cytokines in macrophages via the TLR signaling pathway. This finding was analogous with the degradation of IκB and translocation of NF-κB into nucleus in RAW264.7 cells after exposure to the purified polysaccharides. Taken together, these results suggest that insect-derived polysaccharides have immunomodulatory potential through activating the host's innate immune system, thus allowing it to be a potential immunomodulatory for implementation as functional food supplement in aquaculture or livestock industry.

To elucidate the efficacy of insect-derived polysaccharides as an immunomodulatory substance in a wider perspective, Japanese medaka (*Oryzias latipes*), a teleost model, were fed with dietary silkrose-BM and silkrose-AY and then artificially infected with *Edwardsiella tarda*, a common bacterial disease in finfish, by immersion. Dietary silkrose-BM (10, 100, 1000 ng/g) and silkrose-AY (10 ng/g) significantly improved the survival of fish and reduced the number of *E. tarda* in the kidney of medaka after

infected with *E. tarda*. To begin to understand the underlying mechanism of immunomodulatory activity of silkrose-BM and -AY in teleost, transcriptome profiling approaches using microarray analysis and RNA-seq technology were conducted in the intestine and liver of medaka. Gene ontology and pathway enrichment analysis revealed that dietary silkrose-BM and -AY evoked the expression of various genes with putative role in pathogen recognition, complement and coagulation cascade, antimicrobial peptides/enzymes, activation of phagocytosis/opsonization, and epithelial junctional modification. Similar to the results in mammalian macrophage, silkrose-BM and -AY also capable to induce the expression of various cytokine-cytokine receptor interactions via TLR/NF-κB signaling pathway in teleost. These findings thus provide fundamental information to help understand the molecular mechanism of bacterial protection resulted from dietary supplementation of insect-derived polysaccharides in teleosts.

Further application of dietary silkrose-BM was conducted using *Litopenaeus vannamei* to expand our knowledge on the efficacy of insect-derived polysaccharides as an alternative disease control in shellfish species. Silkrose-BM was chosen in this study due to its unique advantage in terms of availability, as they are a by-product derived from silk production, suggesting a possible synergy between the aquaculture and sericulture industries in the future. The survival of *L. vannamei* after infection with *Vibrio penaecida*, a bacteria that caused vibriosis, was significantly improved by dietary silkrose-BM with doses at 0.0125, 0.25, and 5 μg/g. Analogous with the previous experiments in medaka, transcriptome profiling using RNA-seq was conducted to understand the effects of dietary silkrose-BM in crustacean at transcriptional level RNA-seq profiling resulted in the up-regulation of spätzle genes, a cytokine that crucial for the activation of Toll receptor in invertebrates, suggesting involvement of Toll signaling pathway in the immunomodulatory activity of silkrose-BM in shrimp. These results together with the previous findings on teleost and mammalian suggest that insect-derived polysaccharides might enhance the host innate immune system via Toll/Toll-like signaling pathway in mammalian, teleost, and crustaceans.