



HOKKAIDO UNIVERSITY

Title	Understanding Global Aquaculture Microbiome Trends Toward Constructing Sustainable Local Smart Aquaculture [an abstract of dissertation and a summary of dissertation review]
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学位論文内容の要旨

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学 位 論 文 題 目

Understanding Global Aquaculture Microbiome Trends

Toward Constructing Sustainable Local Smart Aquaculture

（地域養殖の高度化に向けた世界の養殖マイクロバイオーームへの理解）

The objective of this study is to enhance our comprehension of the global aquaculture microbiome to be considered for implementing it into Smart Aquaculture Initiative in Indonesia. The PhD thesis comprises three chapters that investigate the microbiome status of economically valuable aquaculture species across different regions, with a specific emphasis on Asia, the leading global hub for aquaculture production. It will be achieved through microbiome analysis utilizing the Illumina platform. The analysis involved diverse sample datasets obtained from both public databases and laboratory research. The selected aquaculture products represent the most significant categories of aquaculture commodities for Indonesia.

In Chapter 1, *Litopenaeus vannamei* shrimp was chosen as one of the most important aquaculture commodities around the world, including Indonesia. Shrimp aquaculture is a thriving industry, but it is also inherently risky. All the samples were chosen around Asian countries as the highest shrimp producers compared to others. A total of 128 healthy shrimp and 76 diseased shrimp data of 16S rRNA amplicon sequencing taken from Asian countries were retrieved from the public database, and comprehensive analyses were performed. In comparison to life stages and shrimp health conditions that affect shrimp microbiome alteration, geographic location is one of the most influential determinants of shrimp *L. vannamei* microbiome diversity. *Rhodobacteraceae*, *Enterobacteriaceae*, *Cellulomonadaceae*, and *Planctomycetaceae* were more abundant in shrimp microbiomes than in the shrimp ponds. *Rhodobacteraceae* abundance can indicate the overall health of shrimp in all life phases. Several bacteria were discovered as biomarkers in water shrimp pond microbiome. Likewise, several probiotic candidate bacteria have been obtained which are adjusted to the age stage of the shrimp and several bacteria have been identified as bioindicators, which are suspected to be closely related to the occurrence of shrimp disease outbreaks in many countries. *Fusobacteriaceae* and *Vibrionaceae* were the most prevalent in the WSSV-shrimp microbiome. AHPND shrimp had more unclassified families of *Mollicutes*, *Desulfobulbaceae*, and *Sphingomonadaceae* than healthy shrimp. In WFS-infected shrimp, there was an increase in unclassified *Nitrospiraceae*, unclassified *Mb NB09*, and unclassified *Phycisphaerales* in shrimp larvae. *Vibrionaceae* dominated WFS outbreaks in both juvenile and adult shrimp. This study is the first step towards developing a more rapid, accurate, and effective early detection method for disease problems in the aquaculture industry which I call “Smart Aquaculture Initiative”.

In Chapter 2, I examined the gut microbiome condition of some important fish species in Indonesia. The species chosen represented different kinds of fish-feeding behavior. This study will advance our understanding of the microbiome of the host, establish a relationship between various fish feeding habits and the associated gastrointestinal microbes, and offer guidance on how to maximize production in cultured fish by accommodating the dietary needs of each fish species. A total of 84 data sets the Illumina platform for 16S rRNA gene sequencing from four fish, milkfish, tilapia, tiger grouper, and Yunlong grouper, were retrieved from the public database, and

test the hypothesis. *Vibrionaceae* is strongly associated with fish species, like milkfish, that have a higher consumption of plant protein. On the other hand, *Peptostreptococcaceae*, *Fusobacteriaceae*, and *Clostridiaceae* are linked to fish that consume a significant amount of animal protein. Although tilapia is categorized as an omnivore, its gut microbiome tends to be more similar to that of carnivorous fish than herbivorous fish. *Cetobacterium somariae* was also associated with carbohydrate degradation and fermentative processes, making it a potential probiotic candidate for all species, especially for milkfish and tilapia. In the end, our research revealed that fish, especially when considering the gut microbiota, offer a more suitable model for studying humans compared to mice.

In Chapter 3, sea urchin as a bioindicator of coastal environmental changes in the global warming era and is also a model organism in developmental biology and evolution. Due to the depletion of wild resources, new aquaculture techniques for improving stocks have been well studied in Japan. By using metagenomic approaches including meta16S and shotgun metagenome sequencings, the structures, functions, and dynamics of the gut microbiome of *M. nudus* and *S. intermedius*, related to both habitat environment and host growth, were studied with a non-destructive individual methodology for the first time. Firstly, a broad meta16S analysis revealed that at the family level, *Psychromonadaceae* and *Flavobacteriaceae* dominated in these sea urchins, which is a unique feature observed in species in Japan. Our findings revealed that these gut microbial structures were affected by diet rather than rearing environments and host species. Secondly, meta16S analysis of microbial reads related to *M. nudus* growth revealed that at least four Amplicon Sequence Variant (ASV) affiliated to *Saccharicrinis fermentans* indicated contribute to the growth of sea urchins *M. nudus* and *S. intermedius* through their N₂-fixing ability. These findings provide new insights on the structure function relationship of sea urchin gut microbiomes beyond previously reported nitrogen fixation function in sea urchin in 1950s; this study discovered a nitrate reduction function into ammonium for the growth promotion of sea urchin.

Finally, the advancement of bioinformatics technology holds tremendous potential in driving the growth of global aquaculture production. With a deeper understanding of each aquaculture product and the integration of modern information technology, it sets the stage for significant advancements in the industry. The integration of aquaculture's fundamental principles with informatics technology and Artificial Intelligence (AI) is poised to revolutionize the future of aquaculture development.