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学位論文内容の要旨

博士の専攻分野の名称：博士（水産科学）　　氏名：YU JUANWEN

学 位 論 文 題 目

Characterization of pioneer microbiomes associated with *Apostichopus japonicus*: Insights into host-microbe interactions and sustainable aquaculture

（宿主と微生物の相互作用と持続可能な養殖に向けての洞察）

Sea cucumbers (Holothuroidea) are marine organisms known for their commercial value and unique biological characteristics. They play important roles in marine ecosystems as bioturbators, contributing to sediment mixing and nutrient cycling. Sea cucumbers possess intriguing biological phenomena such as autolysis, aestivation, evisceration, and remarkable regenerative capacities, making them excellent model organisms for studying organ regeneration and understanding the evolutionary context of deuterostomes.

Recent advancements in sequencing technologies and the holobiont and hologenome concepts have revolutionized our understanding of host-microbiome interactions. The holobiont concept recognizes the host organism and its associated microbial communities as a collective entity, acknowledging the significant influence of microbes on host biology. Exploring microbiomes has become crucial in unraveling the role of microorganisms in various aspects of host biology, including development, growth, health, and disease. The role of pioneer microbes in the early life microbiome is particularly crucial in shaping later microbiomes and impacting host development and health. These pioneer microbes contribute to nutrient production, immune system maturation, and the prevalence of diseases and disorders. Understanding the role of pioneer colonizers provides insights into holobiont assembly and the long-term effects of early life microbiomes on overall well-being.

With the increasing attention on host-associated microbiomes, the study of microbiomes in sea cucumbers has gained significance due to their high commercial value and the challenges associated with their sustainable aquaculture. Investigating the microbial communities associated with sea cucumbers can provide valuable insights into their growth, health, and disease resistance. Although previous studies have explored the effects of gut microbiomes on sea cucumber growth and the dynamics of microbiomes during gut regeneration, there is still a knowledge gap regarding the establishment of microbiomes during larval development, the composition of the core microbiome, and the occurrence of diseases in sea cucumber holobionts.

Aims of this study is to address these knowledge gaps by assessing the processes of microbial colonization and the establishment of a healthy core microbiome in sea cucumber holobionts. In Chapter 1, I focused on establishing a laboratory breeding system to collect fertilized eggs and larvae at different developmental stages of sea cucumbers. Significant alterations in microbial community structure are observed, particularly in the late auricularia stage, indicating a correlation between developmental stage and microbiota composition. Additionally, a total of 257 strains, including potential probiotics, are isolated from sea cucumber larvae across various developmental stages. These findings shed light on the dynamic changes in the microbiota during early life stages and suggest that studying the interaction between pioneer colonizers and host sea cucumbers may contribute to the development of sea cucumber aquaculture.

In Chapter 2, I investigated normal and gut-regenerated fecal samples from sea cucumber adults to isolate bacterial strains. A total of 153 strains belonging to four genera are identified. A comparison between the microbiomes of wild and normal specimens reveals potential second pioneer colonizers in gut-regenerated

individuals. Furthermore, a meta16S analysis successfully isolates 115 additional amplicon sequence variants (ASVs), providing insights into the proportion of cultivable bacteria. These findings contribute to our understanding of the microbiome reassembly during gut regeneration in sea cucumbers.

In Chapter 3, I focused on metagenomic analyses to assess the core and stage core microbiome during larval ontogenesis in sea cucumbers. The detection of an early life core microbiome, including pioneer core microbes, suggests a stepwise establishment of the microbiome related to ontogenesis and feeding behavior. Notably, the abundance of genome sequences from the growth-promoting bacterium *Sulfitobactor* strain BL28 is detected in the metagenomic sequence pool obtained from early-life sea cucumber stages. These findings underscore the critical role of understanding the core microbiome and associated functions during early life stages in marine invertebrates, as it offers avenues for enhancing growth, immunity, disease resistance, and holobiont maintenance.

In Chapter 4, the occurrence of intestinal atrophy disease in sea cucumber larvae is reported for the first time. Metagenomic analysis is employed to elucidate the differences in the microbiome of atrophy larvae. The potential pathogenic bacterium *Tenacibaculum* is found as being associated with the development of intestinal atrophy disease. The study also highlights the significant role of rearing seawater in shaping the microbiome of atrophy larvae and inducing potential pathogenicity. Further research into the mechanisms and pathogenesis of this disease is necessary for the sustainable development of the sea cucumber aquaculture industry.

Overall, this study provides a deeper understanding of the complex interactions between sea cucumber hosts and their microbiomes. The significance of early-life microbiomes in holobiont assembly is highlighted, emphasizing the importance of microbiome establishment for host development and health. Furthermore, the identification of core microbiome taxa and their functional implications offers a foundation for monitoring and managing the health and ecological status of sea cucumber populations.