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学 位 論 文 題 目

Diversity of Vibrios in the Coral Reef Ecosystem of Ishigaki Island, Japan

(石垣島周辺海域のサンゴ生態系におけるビブリオの多様性)

Coral reefs are productive, diverse and dynamic ecosystems, but are also fragile as their sustainability and resilience are affected by human activities either directly or indirectly. As this ecosystem is host to an abundant variety of microbes, coral-microbes interaction can benefit the host in various ways including photosynthesis, nitrogen fixation and production of antimicrobials. However, coral reefs are in steep decline worldwide due to the combined effects of various stressors such as global warming, pollution, overfishing and infectious diseases. Rising seawater temperature is one of the biggest threats affecting coral health. It is also linked to increasing coral diseases such as bleaching events. Ishigaki coral reef ecosystems are known to be the largest and the most diverse reef ecosystems in Japan, but are currently vulnerable to tremendous stressors including rises in seawater temperature and eutrophication. Some knowledge on reef declining stressors in Ishigaki corals has been accumulated, but less so with regards to the diversity of potential coral pathogens. Despite several vibrios being normal inhabitants of reef systems, a couple of these bacteria act as opportunistic pathogens. Certain environmental conditions influence the emergence and prevalence of these opportunistic vibrios by enhancing proliferation as well as triggering the activity of virulence factors that ultimately leads to coral-vibriosis. In this era of global warming, the surrounding seawater of coral reefs is one of the most important ecological niches as well as heat carriers. It is worth investigating the evolution of spatiotemporal vibrio dynamics in the surrounding waters of the coral reefs for the conservation of fragile coral reefs. The aims of this study are 1) to elucidate the spatiotemporal diversity dynamics of vibrios in the Ishigaki coral reef system, and 2) to understand environmental drivers affecting vibrio diversity. I have also described new *Vibrionaceae* taxa in this study.

Firstly, a three-year survey was performed to elucidate vibrio diversity using a curated *pyrH* gene sequence set. The *pyrH* gene set allows us not only to affiliate vibrio isolates to known described *Vibrionaceae* species or currently unknown new species candidates but also to elucidate the structure of regional populations of specific species and how they have diverged. The findings of this study demonstrate that the vibrio community of the coral surrounding seawater consists of at least 22 known described species and 12 potential novel species candidates (26 isolates) which is likely to be a more diversified vibrio community compared to those reported from tropical and temperate coral reef environments. This study also demonstrates the presence of most of the globally recognized opportunistic potential coral-pathogenic vibrios (*V. owensii*, *V. harveyi*, *V.*

alginolyticus) including the notorious known coral pathogen *V. coralliilyticus*. Interestingly, significant positive correlation was observed between rising seawater temperature and the abundance of *V. campbellii* ($r=0.62$; $P<0.05$) whereas the opposite was observed for *V. owensii* ($r=-0.58$; $P<0.05$) and the C6 group of *V. hyugaensis* ($r=-0.62$; $P<0.05$). AdaptML-based microhabitat differentiation revealed that *V. harveyi*, *V. campbellii*, and *P. rosenbergii* populations were less-ecologically distinctive whereas *V. astriarenae* and *V. ishigakensis* were ecologically diverse. In addition, the global coral pathogen *V. coralliilyticus* was not correlated to the assessed environmental drivers.

Secondly, this study also disclosed several phylogenetically unique vibrios from the Ishigaki reef systems. Applying the most recent advanced microbial taxonomic approaches fulfilling monophyly, genetic, and phenotypic cohesion, these strains are proposed as a new genus in *Vibrionaceae* containing two new species *Thaumasiovibrio occultus* gen. nov., sp. nov., and *Thaumasiovibrio subtropicus* sp. nov. Both of the described species, *T. occultus* and *T. subtropicus* are catalase negative and showed green colonies in TCBS which are their important distinguishing criteria from the existing genera in the *Vibrionaceae* family. The Ishigaki coral reef ecosystem is also a hot spot for the study of vibrio evolution. In addition to the *Thaumasiovibrio*, 11 other potential new species candidates have been isolated. They are related to *V. harveyi*, *V. campbellii*, *V. communis*, and *V. ponticus*.

Among microbes, vibrios represent one of the best studied models for the ecology and evolution of bacterial populations in nature. The detections of relevant environmental drivers are crucial in predicting the future behavior of vibrio communities and/or populations in stressed coral reef ecosystems. I gained fundamental knowledge on spatiotemporal vibrio dynamics both at community and population levels in the Ishigaki reef ecosystem along with their relationship to environmental drivers. This study could be an important gateway for further research in coral resilience and microbial diversity change in the Ishigaki coral reef ecosystem, which has experienced catastrophic damage.