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学 位 論 文 題 目

Pangenome analyses of Halioticoli, Ponticus, and Splendidus clades in the family *Vibrionaceae*
(ビブリオ科の Halioticoli, Ponticus および Splendidus クレ ードのパンゲノム解析)

Vibrionaceae is one of the most diverse bacteria, plays an important role in the mineral cycle, pathogenicity, ecology, and systematics. Currently, over 190 species in the family *Vibrionaceae* have been described and classified into over 9 genera, in which the number of species has doubled compared to the most recent vibrio evolutionary by Sawabe *et al.* 2014. A total of 24 clades were proposed in the family *Vibrionaceae* in 2014, however, inter- and intra-clade genome features have never been fully studied due to delays of complete genome sequencing. Members of *Vibrionaceae* are often associated with humans and animals in different relationships, which include symbionts and pathogens. Some of them have been well-studied, such as *Aliivibrio fischeri* (a squid light organ symbiont) in Fischeri clade, *V. cholerae* (a well-known human pathogen) in Cholerae clade, and *V. harveyi* (a broad fish pathogen) in Harveyi clade. However, many members remain poorly understood because of the lack of genomes. For example, Halioticoli clade species (mostly abalone associated), Ponticus clade (an emerging fish pathogen included), and Splendidus clade (most diverse clade with pathogenicity). Therefore, the aim of this research is to further update the evolutionary units of *Vibrionaceae* and perform the first pangenome analyses for Halioticoli, Ponticus, and Splendidus clades using complete genomes.

In this study, I did have the complete genome sequences for 45 *Vibrionaceae* species and performed the genome-based analyses for these less studied clades. In **Chapter 1**, the up-to-date molecular phylogenetic analysis of the family *Vibrionaceae* using 191 genomes including 27 newly obtained ones was updated. Results described a total of 51 distinct clades including 21 newly defined ones (Vibrio Clade 3.0), and three species were likely

misidentified. Compared to other gene sets, the 8-HKGs MLSA is still an effective and reliable tool for delineating new species, monophyletic groups in *Vibrionaceae*. Finally, the success of identification in *Vibrionaceae* using MAGs showed the potential of the 11-RPGs set in classifying or identifying species candidates in MAG or SAG applications. In **Chapter 2**, two strains C4III282^T and C4III291, isolated from seawater collected from a site off the Taketomi coral reef, represent a new species in the Ponticus clade, based on 16S rRNA gene phylogeny, 8-HKGs MLSA, ANI, *in silico* DDH, and pan-genome analysis using complete genomes. The name *Vibrio taketomensis* sp. nov. is proposed with C4III282^T (CAIM 1928^T = DSM 106943^T = JCM 33434^T) as the type strain. In **Chapter 3**, the complete genome sequences of type strains of all current Halioticoli clade species were presented and the first genomic analyses for this clade to evaluate their ecogenomics, evolutionary history, and possible biotechnology applications were performed. Results revealed five sub-clades (SCs) in the Halioticoli clade, in which, SC1 to SC4 consist of symbiotic species, and SC5 consists of only one planktonic species. It appears that genome expansion encoding more carbohydrate metabolism occurred during symbiotic as a gut-living to planktonic environments. The planktonic species acquired more abilities to utilize a variety of carbohydrates for surviving in the environment while symbiotic species were evolved to specialize in macroalgae utilization. In **Chapter 4**, 19 true members of the Splendidus clade were defined except *V. cortegadensis* CECT 7227^T. Molecular phylogeny revealed three SCs with the 15 most closely related members as SC1. Comparative genomic analyses and complete prediction of virulence factors suggest that a T2SS system may be involved in the virulence mechanism and environmental fitness of the Splendidus clade. AI-2 and CAI-1 quorum sensing systems are likely to be necessary for the virulence of Splendidus clade pathogens.

All these findings will pave the way for a better understanding of not only the diversity and evolution of *Vibrionaceae* but also the exploration of bacteria-host interactions, which could contribute to social and commercial achievements in the future.