MCEF21101 Population genomics of coral-associated denitrifying bacteria along nitrate **gradients in Hong Kong waters**

1. Executive summary

The urbanized development of Hong Kong has led to significant eutrophication in local waters, jeopardizing the health and survival of corals and coral reefs. Corals evolve a symbiotic association with photosynthetic algae and a variety of functionally varied prokaryotic microbes. Denitrifying bacteria are prokaryotes that can transform nitrate and nitrite into nitrous oxide and dinitrogen gas. Recent research indicated that denitrifying prokaryotes were prevalent in coral holobionts, typically characterized by a simple structure and predominance of a few genera, including *Labrenzia*, *Ruegeria*, and *Ralstonia*.

We selected *Ruegeria* as our research subject and isolated several *Ruegeria* from Hong Kong corals, performing the whole genome sequencing, and categorizing them into distinct populations by population genomics. We have annotated all *Ruegeria* strains possessing denitrification genes and discovered that over 80% of these strains possessed a complete repertoire of denitrification genes that may facilitate this process. Consequently, a synthesis of *parC* amplicon and enrichment analysis for corals sampled in Hong Kong reefs along the nutrient gradients enabled us to discover five denitrifying *Ruegeria* populations that were markedly enriched in the western reefs, which we designate as “specialist denitrifying *Ruegeria* populations”. Experiments utilizing the 15N stable isotope labeling demonstrated that representative specialists denitrifying *Ruegeria* populations exhibited a higher denitrification activity compared to representative generalist denitrifying *Ruegeria* populations, with a larger amount of dinitrogen gas as the product.

This study has significant ecological implications for coral reef conservations in Hong Kong. It elucidates the presumed function of *Ruegeria* in enhancing coral fitness through microbial evolution and identifies a group of specialist denitrifying *Ruegeria* populations with exceptionally high denitrification activities. Isolates from specialist denitrifying *Ruegeria* populations may serve as potential probiotics to enhance coral resistance to local eutrophication stressors.