Cross-SRA Collaboration within SBS
Supplementary Material

Project title: *The Environmental Framework for Tackling Antibiotic Resistance.*
Applicants: *Dr G. Panagiotou (Cell Biology), Dr A. Yan (Cell Biology), Dr H. El-Nezami (Food for Health), Dr D. Baker (Ecology & Biodiversity).*

General Research Objective: In Hong Kong, we are afforded the rare opportunity to assess the relationship between cryptic biodiversity, ecosystem function and human health owing to distinct punctuated gradients in water quality and foundational species richness. Standardized passive samplers such as Autonomous Reef Monitoring Structures (ARMS) will be used with next-generation sequencing (NGS) technologies and plate cultivations to characterize marine prokaryotic biodiversity in Hong Kong. Specifically, DNA shotgun and plate-based metagenomics will provide an unprecedented level of resolution for quantifying biodiversity (species richness) and ecosystem function (e.g. metabolic pathways, pathogenicity, antibiotic resistance, biogeochemical cycling, etc.).

Aims: Besides characterizing the prokaryotic diversity of the sampled environments our goal is to understand in **what degree the distances from human activities influence the abundance and dissemination of pathogens and antibiotic resistant genotypes**. More specifically, this project aims to contribute in establishing the most impactful policies that limit the dissemination of antibiotic resistance by (1) **uncovering which marine microbial communities participate more in antibiotic resistance gene exchange;** (2) **is there a core resistome in the different sampling locations that does not depend on human activity?**
Furthermore we believe that our proposal is in-line with research initiatives from other members of the Faculty of Science, e.g. Prof. Kenneth Leung is submitting in this round a CRF application with exactly the same research objectives as presented here, the antibiotic resistance, but different sampling locations (wastewater treatment facilities), therefore synergies between the different groups could become a competitive advantage for our school.

Deliverables: (1) One publication in an international peer reviewed journal; (2) A new MPhil student, Mr Jiarui Chen (GPA=3.4/4), will be hired using a Type B place in April 2016 and he will be co-supervised by Dr Panagiotou, Dr Yan and Dr Baker; (3) A CRF application will be submitted and the data generated in the framework of the current proposal will be used to support the CRF. The title and abstract of the CRF application is attached below.
Based on the amount requested here (~200,000 HKD) it is our belief that the deliverables of the project are sufficient and will be completed on time (June 2017).

WP 1 – PI Dr D. Baker: Dr Baker's team has deployed 12 ARMS in 4 sites along a water quality gradient in May 2015 (Centre island, Che Lei Pai, Port Island and Tung Ping Chau) and we plan to remove them for analysis in May 2016. Seawater in the container in which the retrieved ARMS device was placed prior to disassembly will be filtered through different types of sieves that vary from 2 mm to 100 um. This will result in 3 sieved fractions (>2mm, 500 um to 2mm and 106 um to 500 um). Species that are retained by the largest sieve (>2mm) will be sorted morphologically and preserved in 95% ethanol for further processing. They will then be sent for DNA barcoding and taxonomic assignment. We estimate the sample size of the >2mm sieved fraction to be between 80 and 100 per ARMS. Following this, species retained by the 500 uM sieve and 100 uM sieve will be preserved in 95% ethanol for DNA metagenomics. All samples will be stored at -20 degree Celsius.
Marine sediment is one of the largest biospheres in the world (Whitman et al., 1998) and is known to be home to large microbial populations. As they provide a valuable source of the diversity and presence/absence of pathogens, it will be one of the core research methodologies of our project to quantify the prokaryotic diversity and ecosystem function in the marine environment. Benthic sediment samples will be collected from the 4 sites using

established protocols (Orsi et al., 2013). Sediments will be collected using a Van Veen Grab. 50g of the sample will be fixed with 10ml of 4% of formaldehyde and stored at -20ºC for DNA metagenomics.

WP 2 – PI Dr G. Panagiotou: Metagenomics on sediment samples will be done using established protocols (Sun et al.,2013). MoBio PowerSoil DNA isolation kits will be used to extract genomic DNA from the sediment samples after which bacterial community composition will be determined using shotgun metagenomic sequencing. The distribution of global and unique taxa will be studied across Hong Kong's waters using Illumina NGS sequencing technologies. Taxonomic assignments and defining the resistome reservoir of the microbial communities will be based on appropriate reference databases. Furthermore, the group has long experience in antibiotics resistance in the human gut microbiota therefore in-house databases and scripts will also be used.

- Quality Check – Reads will be trimmed if sequences (1) were shorter than 250 bp (2) had more than two mismatches per primer sequence (3) had any ambiguous base call and (4) had at least one homopolymer region longer than 8 bp.
- Alignment/Assembly – Reads will be assembled after checking for stop codons, frame shifts and deletions. These two steps maximize the reliability of the sequence assembly and is vital for data analysis.
- Taxonomic assignments/characterization – Sequence data set obtained will be run through BLASTn searches with GenBank as a reference database. For bacterial and viral sequence classification, MetaPhlAn/BWA can be used for alignment and assembly and subsequently to map with suitable reference databases. To further investigate results of potential pathogenic bacteria found in the marine environment, sample sequences can be compared with virulent plasmid(s)'s sequences.
- In the absence of a direct match, which is likely in MarineGEO-Hong Kong, de novo assembly will be used and operational taxonomic units (OTUs) will be assigned to higher taxonomic levels.

WP 3 – PI Dr A. Yan: Cultivations for characterizing the resistant phenotypes will be performed as follows: Five grams of sediments will be homogenously resuspended in 50 mL of 1X PBS that had been pre-reduced with resazurin (0.1 mg/mL). Ten-fold serial dilutions will be performed performed to 10^{-9}. One hundred microliters of the serial dilutions will be plated in 2 replicates on GAM agar containing one of 16 antibiotics and GAM agar containing no antibiotics. The selected antibiotic concentrations will be based on Eucast data (http://mic.eucast.org/Eucast2/) of commensal gut bacteria, and will be aimed for 2x higher than MICs. The average CFU/mL plate counts will be determined using the countable plates (25-250 colonies). The percentage of resistant bacteria grown on each antibiotic at specific sampling locations will be determined by dividing the average colony counts from the antibiotic plates by the control plate colony counts. The relative resistance level for each taxon is defined as the ratio of the relative abundance of the taxon in each antibiotic plate to the relative abundance of the taxon in each control plate for each sampling location. To test whether certain taxon has enhanced resistance over time, the resistance levels among different plates for this taxon (e.g. all beta-lactam plates) will be compared using Wilcoxon signed-rank test.

Collaborative Research Fund:
Project Title: MarineGEO-Hong Kong: Towards an Understanding of Marine Biodiversity and Ecosystem Function

Summary: With Hong Kong's signing of the Convention on Biological Diversity (CBD), it is now a top priority to improve our understanding of biodiversity (species richness) and ecosystem function (productivity, nutrient cycling, etc.). However, current methods of marine environmental monitoring in Hong Kong are focused on in situ visual surveys and collections

of conspicuous (>2mm) macrofauna. With the exception of plankton, there are few studies that have assessed the "cryptic " (<2mm) biodiversity of Hong Kong's benthic marine environment. This represents a major knowledge gap given that more than 500,000 marine species (>60%) are cryptic and unknown to science (coml.org). Although difficult to see, these species are often abundant and perform important functions in the environment. By ignoring these species, environmental policy decisions are based on a limited and fragmented understanding of biodiversity and offer little in regard to quantifying ecosystem functioning.

To address this gap, this proposal seeks support from the CRF to partner Hong Kong with an exciting global marine biodiversity initiative, MarineGEO.

The importance of biodiversity is not limited to discovery. Meiofaunal and microbial assemblages perform essential roles in marine ecosystems and constitute the base of the marine food chain. These roles make them especially important for human health and wellbeing . In a mega-city such as Hong Kong, humans live in close association with the ocean while nearly 3 million m3 of treated wastewater gets discharged into the coastal marine environment every day. The microbial and chemical constituents of these effluents represent a major risk to human and environmental health. It is therefore important to conserve structurally and functionally diverse marine communities to suppress the emergence of pathogenicity, virulence factors and ultimately the prevalence and severity of disease. In Hong Kong, we are afforded the rare opportunity to assess the relationship between cryptic biodiversity, ecosystem function and human health owing to distinct punctuated gradients in water quality and foundational species richness. Using a new international framework - MarineGEO, this proposal seeks to create new synergies by integrating conventional taxonomy-based approaches with improved quantitative, process-level understanding of biodiversity and ecosystem function. Standardized passive samplers such as Autonomous Reef Monitoring Structures (ARMS) will be used with next-generation sequencing (NGS) technologies to characterize marine prokaryotic and eukaryotic biodiversity in Hong Kong. Specifically, DNA metagenomics and (mRNA) metatranscriptomics will provide an unprecedented level of resolution for quantifying biodiversity (species richness) and ecosystem function (e.g. metabolic pathways, pathogenicity, antibiotic resistance, biogeochemical cycling, etc.). As a result, MarineGEO-Hong Kong will provide big data from the cutting edge of science to construct a new foundation for environmental policy decisions and new baselines from which future long-term monitoring can be used to benchmark management decisions while also anticipating the impacts of global climate change.