Form 3

Annual Report

LaMer, Ehime University

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To Director of LaMer

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Position : Senior Lecturer
Name in print _____

Project title

Antibiotic resistance genes in marine aquaculture sites in Peninsular Malaysia

Members of project

Name	Affiliation	Position	Contribution part
Bong Chui Wei（PI)	Institute of Biological Sciences, Faculty of Science, University of Malaya	Senior Lecturer	Project design, performed the experiments and analyses of results
Contributing Members			
Lee Choon Weng	Institute of Biological Sciences, Faculty of Science, University of Malaya	Associate Professor	Project design and analyses of results
Thiang Ee Lean	Institute of Ocean & Earth Sciences (IOES) University of Malaya	Postgraduate Student	Performed the experiments

LaMer Faculty member in charge **Satoru Suzuki**	Leading Academia in Marine and Environment Pollution Research, Center for Marine Environmental Studies, Ehime, University, Japan	Professor	Provided feedback and discussion on the project design and the outputs of the results Contributed reagents/materials/analysis tools

Aim

To quantify antibiotic resistance genes (tetracycline and sulphonamide) in marine aquaculture.

Procedure

Sampling has been carried out at seven main seafood production districts (Perak, Selangor, Pahang, Kelantan, Penang Island, Malacca and Johor) of Peninsular Malaysia. Water samples were also collected from upstream to downstream of river estuary in Perak including runoff waters from zoo, slaughter house, hospital, aquaculture. Seawater samples were filtered through 0.2 µm polycarbonate membrane filter (Merk Millipore, Germany) to collect the total DNA of natural bacterial assemblages. DNA from the membranes were extracted according to Suzuki et al. (2013). Antimicrobial resistance (AMR) genes: *tetM*, *sul1*, *sul2* and *sul3* were quantified by quantitative PCR (qPCR) using CFX 96 Real-Time system (Biorad, Laboratories, Hercules, CA, USA) in CMES which method has been establised.

Results

We have successfully analyzed the abundance of targeted antibiotic resistance genes (*sul* and *tet(M)* genes) for all the samples within the schedule. Of the all the resistance genes analyzed, *tet(M), sul1 and sul2* were the most abundant, with detected copy number in total assemblages in aquaculture farm, ranging from~10^{-5} – 10^{0}/16S, ~10^{-5} – 10^{-1}/16S, ~10^{-6} – 10^{0}/16S, respectively, whereas *sul3* gene was at very low abundance or not detected in the total assemblage at most of aquaculture farm, with copy number of ~ 10^{-7} – 10^{-4}/16S. This indicates that *tet(M), sul1* and *sul2* are ubiquitous in bacterial communities in marine aquaculture. Same pattern of *sul* and *tet(M)* genes were observed in Perak river estuary. The copy number of *tet(M)* and *sul* (*1 & 2*) genes were relatively higher compared to the previous studies in China and the Philippines (Gao et al., 2012; Suzuki et al., 2013; Xiong et al., 2014; Xiong et al., 2015; Niu et al., 2016;). However, the relative abundance for *tet(M)*

and *sul* (*1 & 2*) genes are lower compared to wastewater treatment in Norway (Lath et al., 2014).

Among the aquaculture farms, of all the farms 92% of aquaculture farms were detected both *sul1* and *sul2*, followed by *tet(M)* (86%) and sul3 (69%). The co-existence of *sul* genes, combination of *sul1* and *sul2* were the most frequent which detected in 30 farms, whereas, the co-existence of *sul1* and *sul3 (n=24)*, *sul2* and *sul3 (n=23)*, *sul1*, *sul2* and *sul3* (n=23) were lower. Overall, the frequency of antibiotic resistance genes was *tetM>sul2>sul1 >sul3*.

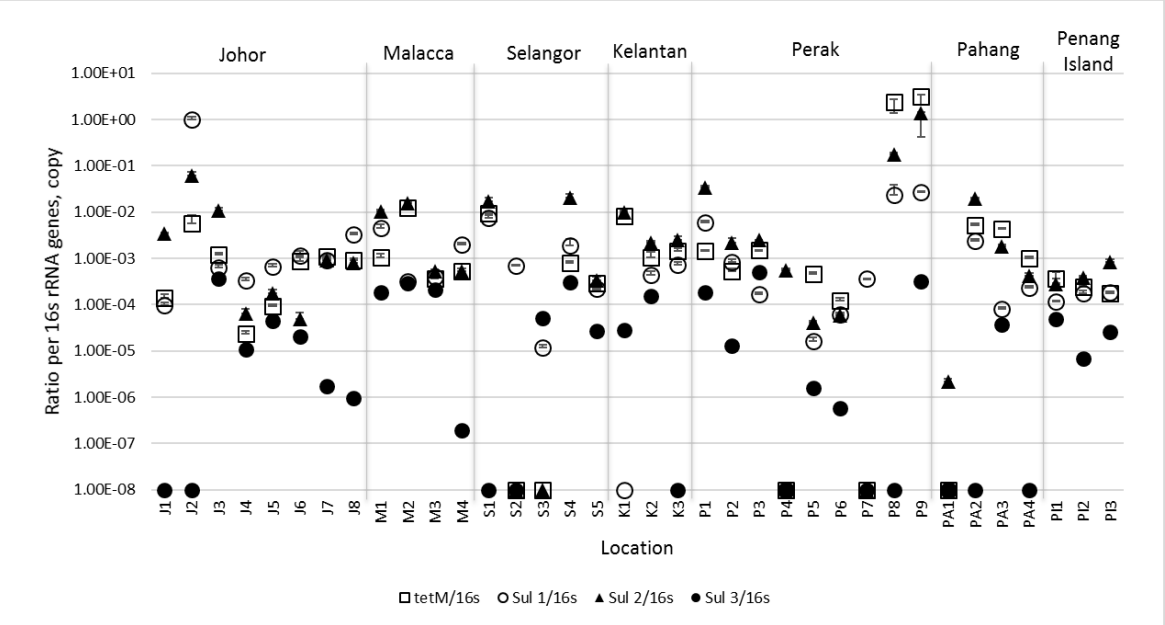

Figure 1. The abundance *of tet(M), sul1, sul2* and *sul3* genes in total assemblages.

Perspectives in Future

Unfortunately, we are unable to analyses the antibiotic residues in marine aquaculture, due to the time constraints. In future, we hope to have an opportunity to learn the techniques in quantifying antibiotic residues in the water at CMES so we could have more insights into the relationship between antibiotic residues and antibiotic resistance genes (ARGs) in marine aquaculture. Further study, to explore the abundance and diversity of ARGs in both the cultured and uncultured community in marine aquaculture and their correlations between antibiotic residues will definitely help us to obtain a better picture of the environmental reservoirs of antibiotic resistance and their potential impacts on public health.

List of Publication

Two manuscripts are in preparation:

a. Sulfonamides residues and sulfonamide resistant bacteria and their genes in Taiping river estuary, Perak
b. Occurrence and distribution of antibiotic resistance gene in marine aquaculture.

Conference Presentation

Lye, YL, Bong, CW, Zhang RJ, Zhang G, Satoru S & Chai, LC (2017). Sulfonamide residues and resistance genes in Taiping river estuary, Perak. The Third Xiamen Symposium on Marine Environmental Sciences, 9-11 Jan 2017, Xiamen University, China