UM HIR STUDENTS’ SEMINAR
Date : 21st February 2013 (Thursday)
Time : 3.00 pm
Venue : Seminar Room, Aras 1, Bangunan High Impact Research (HIR)

1. Detection of Unusual Long Chain N-Acyl Homoserine Lactones (AHL) and Quorum Quenching Activity from Bacteria Isolated from Diseased Tilapia Fish

Name : Xinyue Chan
PI Name : Dr. Chan Kok Gan

Abstract

Bacteria communicate with each other in an enclosed environment by the production of signaling molecules. This cell -cell communication is termed quorum sensing (QS) that relies on QS molecules for the regulation of the bacteria group behavior including the virulence factor genes expression. QS has been regarded as an important aspect in aquaculture.

The high demand of tilapia fish by domestic and nationwide market has made Tilapia fish farming an important aquaculture industry in Malaysia. In 2009, a fish farm in Terengganu (Malaysia) was badly hit by an endemic disease outbreak which killed more than 50 % of the tilapia fish and the causative agent was suspected to be bacteria. In this study, five potential bacterial pathogens including Bacillus sp. W2.2, Klebsiella sp. W4.2, Pseudomonas sp. W3 and W3.1 and Serratia sp. W2.3 were isolated from the diseased tilapia fish.

With the aid of high resolution quadruple liquid chromatography mass spectrometry, we detected the presence of unusual long chain N-(3-oxohexadecanoyl)-homoserine lactone (3-oxo-C16-HSL) from Pseudomonas sp. W3.1 and N-dodecanoyl-homoserine lactone (C12-HSL) from Serratia sp. W2.3, respectively. This study is the first documentation that shows unusual long-chain AHLS production in Serratia sp. and Pseudomonas sp. isolated from diseased fish whereby production of such long chain AHLS in these bacteria has not been reported elsewhere.

In addition to AHLS, Pseudomonas sp. W3.1 also produced a wide range of Pseudomonas quinolone signalling (PQS) molecules. Proteolytic and haemolytic activity assays have confirmed that Bacillus sp. W2.2, Pseudomonas sp. W3 and W3.1 possessed both activities while Serratia sp. W2.3 showed only proteolytic activity. In contrast to Pseudomonas sp. W3.1, Pseudomonas sp. W3 did not show any quorum sensing properties but possessed quorum quenching activity that inactivated a broad range of AHLS.
2. An Analysis of Marine Metagenomics using Bioinformatic Approach
Name: Ramitha Arumugam  
PI Name: Dr. Lawrence Choo Siew Woh  

Abstract  
Ocean contains plenty of undiscovered valuable microorganisms. There are 2 objectives in this project; to study the diversity of the ocean sample and identify putative Quorum sensing and biocatalyst genes. Here, we use a whole-genome shotgun and metagenomic approach to uncover the microbial diversity in the sea water sample. A total of 6,701,060 reads were generated from the shotgun sequencing. These raw sequences were trimmed and BLASTed against NCBI NR environmental database using the built-in BLAST algorithm of the Cyber infrastructure for Advanced Microbial Ecology Research and Analysis (CAMERA) portal. A total of 24 eubacteria phyla were identified, and of these, Proteobacteria made up the largest division followed by Bacteroidetes and Actinobacteria. Meanwhile, there were 5 phyla of Archaea identified namely Eurkarchaeota, Thaumarchaeota, Crenarchaeota, Korocheato and Nanoarchaeota together with some unclassified Archaea 16S genes.  
Although a high diversity of microorganisms is observed, more sequencing work needs to be done for better understanding on the microbes as the DNA are yet to be sequenced to saturation. Besides the diversity study, we are in the process of identifying putative quorum sensing genes which are known to regulate cell density-dependent gene expression, and biocatalyst genes which are enzyme complex consisting of, or derived from, an organism or cell culture that catalyzes metabolic reactions living organisms in the marine metagenomic sample. The identification of these genes may have commercial values, for example, to overcome the oil pollution at ocean environment.

3. Molecular Diversity of HIV-1 and Surveillance of Transmitted Drug Resistance Variants in Treatment of Naïve Patients in Kuala Lumpur, Malaysia: 5 years after Active Rollout of HAART.  
Name: Lai Yee Ong  
PI Name: Dr. Tee Kok Keng  

Abstract  
Introduction:-  
Free highly active antiretroviral therapy (HAART) was made widely available in Malaysia in 2002. For the purpose of developing treatment guidelines for HIV-1 positive population, it is imperative to investigate transmittable drug-resistant HIV-1 variants among HAART naïve patients. Thus the aims of this study were to assess the prevalence of drug-resistant HIV-1 variants and to identify circulating subtypes among HAART-naïve patients.  

Method:-  
Plasma specimens from N=100 HIV+ HAART-naïve adults were collected between March 2008 and August 2010 and viral RNA was extracted for nested polymerase chain reaction (PCR) and sequenced: codon 1-99 and 1-253 of the protease (PR) and reverse transcriptase (RT) genes, respectively.
PR and RT protein sequences were aligned and the transmittable drug resistance mutations were identified using Stanford database and compared with WHO surveillance list. Phylogenetic reconstruction (HXB2: 2253-3311nt) using neighbour-joining tree based on the Kimura two-paramater nucleotide substitution model with transition-to-transversion ratio of 2.0 implemented in Mega 5.05.

Recombination break point analysis were performed to determine the genotypes and diversity of the viruses using SimPlot 3.5.1 bootscan tool. Bootscanning was performed based on the Kimura two-paramater model with a sliding window of 200 bp in size and 20 bp increment.

Results:-
Based on the WHO consensus guidelines, none of the recruited HAART-naïve patients had any transmitted drug resistance mutations in the PR and RT genes. However, analysis using the Stanford guidelines showed that 35% of the patients had at least one previously reported mutation that may reduce drug susceptibility, protease inhibitors (PI), nucleoside RT inhibitors (NRTI) and non-nucleoside RT inhibitors (NNRTI). The commonly detected mutations that may affect current first line therapy was V179D, which may lead to reduced susceptibility to NNRTI. The predominant circulating HIV-1 genotypes were CRF01_AE (51%) followed by CRF33_01B (17%). Prevalence of unique recombinant forms (URF) was 7%; five distinct recombinant structures involving CRF01_AE and subtype B' were observed, among them a cluster of three isolates that could form a novel circulating recombinant form (CRF) candidate.

Conclusion:-
The prevalence of transmitted drug resistance among treatment naive patients was low in Kuala Lumpur despite HAART rollout 5 years previously. Owing to the high genetic diversity, continued molecular surveillance can identify the persistent emergence of HIV-1 URF and novel CRF candidates with significant epidemiological impact.

ALL POSTGRADUATE STUDENTS, POSTDOCS AND RAs WORKING IN ARAS 1, B.HIR, ARE STRONGLY ENCOURAGED TO PARTICIPATE IN THIS SERIES OF SEMINARS.