Genomic Approaches to Explore Bacterial Diversity in the Tropics

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Despite a growing international interest in preserving biodiversity, many biologists and policymakers tend to overlook the importance of microbial diversity. Yet, microorganisms are integral actors in natural ecosystem and become resources for valuable materials ("biodiamonds") to many industries. Indeed, microorganisms are of central importance to our planet sustainability and it underpins all of biology. A lack of accessibility is one of the main reasons that microbial diversity is poorly appreciated.

The conventional methods to cultivate microorganisms in the laboratory did not reflect the actual diversity in their natural habitats since those methods only demonstrate the presence of cultivable or "culturable" microorganisms. In fact, several lines of evidence suggests that the majority (95-99%) of microorganisms in nature have not been cultured or "unculturable". In addition, systematic for culturable microorganisms, based on morphological or physiological properties are not reliable to show functional groupings, and it has underestimated the diversity of microbial world.

Advances in molecular biology, especially in DNA sequencing, DNA tagging, and PCR, has allowed scientists to determine the number, composition, and distribution of microorganisms, including the non-cultivable ones, in their natural habitats. Molecular systematic based on comparison of ribosomal DNA sequences has revealed three domains of life, i.e. Archaea, Bacteria, and Eukarya, which renew the concept of biological evolution and the study of microbial diversity. The availability of molecular techniques in the study of microbial diversity has not only significantly accelerated our understanding on comprehensive ecology and evolution, but also in our approaches to optimally harvest "biodiamonds" for human welfare.

Bledug Kuwu is an active terrestrial-volcanic-mud pool possesses salinity of 4-8% (w/v) and experience continuous mixing due to the formation and explosion of *giant mud bubbles* by gas pressure underneath the pool. The mud temperature was approximately the same as the surrounding temperature, i.e. 28-32°C. Some preliminary geological studies as well as local legends suggested that Bledug Kuwu and its surrounding areas, which is geographically nested in the middle of Java island, used to be a prehistoric ocean or somehow connected to a marine ecosystem elsewhere. In this study, we grew enrichment cultures from mud samples obtained from Bledug Kuwu, and isolated total DNA directly from the resulting enrichment cultures. The total DNA obtained was used as template to amplify 16S-rRNA genes employing specific primers for Bacterial domain. The 16S-rRNA genes were cloned into a specific vector before they were subjected for DNA sequencing to reveal nearly complete sequence of the 16S-rRNA genes. DNA sequence comparison and phylogenetic analysis indicated that DNA sequence of BK2-1 and BK2-2 clones were closely related to marine prokaryotes available in the database. The closest relative of BK2-1 is Mariana No.1, a *non-culturable* bacterium deduced from 16S-rRNA sequence of soil sample obtained from Mariana trench in Japan sea, the deepest trench on earth. Amplified Ribosomal DNA Restriction Analysis (ARDRA) of 16S rRNA gene library from the mud revealed at least nine different DNA profiles.

BK-05 is a cultivable, moderately halophilic isolate from the mud that showed similarity to the 16S-rRNA

gene of either *Halobacillus halophilus* or *Halobacillus karajiensis*. We isolated the gene/s involved in the biosynthesis ectoine, a compatible solute for osmoprotectant. To isolate these gene/s, primers were designed from the sequence of *Halobacillus halophilus*. These primers were employed to amplify the corresponding genes from BK-05. The PCR reaction yielded one fragment of the expected size, which was cloned and partially sequenced. Sequence analysis for the putative ectoine biosyntesis genes in the plasmid vector (designated as pECTR) revealed the presence of the *ect* genes in the following order: *ectA*, *ectB*, and *ectC*. The deduced partial protein from pECTR forward primer was very similar to the L-2,4 diaminobutyric acid acetyltransferase from *Marinococcus halophilus*, while partial protein sequence from pECTR reverse primer was very similar to the ectoine synthase from *Bacillus halodurans*. We found that the transcription of the *ect* operon is salt dependent. Although there was no *ect* genes expression in the absence of NaCl, the expression of these genes increased linearly with increasing salt concentration up to 2.5 M NaCl.

The results of this study will not only enhance our understanding on the geological formation of Bledug Kuwu or Java island, but will also provide important information for novel genes for biotechnology and *bioprospecting* in this unique habitat.