Doctoral Thesis

Regulation of biofilm formation via phosphorelay and quorum-sensing system in *Vibrio cholerae*

(Summary)

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This research aimed to determine the regulation of the quorum-sensing system regulator HapR and ArcAB two-component signaling system on the biofilm formation of *Vibrio cholerae* under anaerobic condition.

In the first study, the relationship of HapR to the biofilm formation of *V. cholerae*, and the influence of *hapR* sequence polymorphisms and oxygen conditions on the biofilm formation of the pathogen was investigated. Two major *hapR* haplogroups were established. *hapR1* is comprised of environmental non-pathogenic strains while *hapR2* is dominated by cholera-causing strains. Frameshift and terminal deletion HapR variants showed the highest biofilm formation whereas intact HapR variants showed the least. Thus, mutations resulting to either disruption on the transcription factor binding sites or dimerization of the HapR will result to a non-functional biofilm repressor leading to higher biofilm formation.

The strong correlation between the pathogenic *V. cholerae* strains and the *hapR2* haplogroup motivated the second study to develop a simple allele specific molecular marker for the detection of pathogenic *V. cholerae* strains. A common forward primer and an allele-specific reverse primer were designed to detect the pathogenic strains. The AS primers can be used extensively with low to high efficiency polymerases and showed high specificity and high sensitivity with crude or purified chromosomal DNA. Enrichment of seafood samples using APW from 7 to 8 hours is suggested for qualitative assessment of pathogenic *V. cholerae* presence in seafood samples.

The third study then geared towards elucidating the role of the ArcAB system and HapR regulator on the biofilm formation of *V. cholerae.* Construction of isogenic *arcAB* deletion mutants having either functional or non-functional HapR was done. Intact HapR *arcAB* mutants showed reduction in biofilms whereas frameshift and terminal deletion HapR *arcAB* mutants showed variable biofilm formation. The reduced biofilms of Δ*arcA* mutant was not

correlated with growth but a result of reduced expression of biofilm-associated genes. An epistatic relationship between the ArcAB system and HapR on the biofilm formation of *V. cholerae* was speculated and system regulates *V. cholerae* biofilm formation directly through the HapR regulator.

The fourth study established a new regulatory network of the ArcAB system on the biofilm formation through HapR. Gel-shift assay revealed that phosphorylated ArcA can bind to the promoter region of *hapR*. Confirmation of this regulation was done through gene expression of *hapR* and *hapA*. Disruption of *arcAB* has led to an increase in the expression of these genes suggesting that ArcA represses *hapR* transcription.

In conclusion, this research established a new regulatory network on the biofilm formation of *V. cholerae*. The ArcAB system promotes biofilm formation of *V. cholerae* by repressing *hapR* transcription in intact HapR *V. cholerae* strains.