Involvement of Quorum-Sensing Regulator in Biofilm Formation by Pathogenic Bacterium, *Vibrio vulnificus*

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Numerous bacterial species showed capabilities to form surface-associated multicellular structures. The biofilm formation is composed of distinct developmental stages, which include an attachment/adhesion of a single cell, a proliferation toward monolayered coverage, a propagation to aggregated microcolony, a maturation to three-dimensional structure, and subsequently a local degradation (Costerton et al., 1999). The mature biofilm is equipped with extracellular polymeric matrix and internal water-filled channels (Stoodley et al., 2002). This complex architecture can be achieved by differential expressions of several hundred genes, among which the most studied are the genes coding for exopolysaccharide biosyntheses (Friedman and Kolter, 2004) and quorum-sensing components (Zhu and Mekalanos, 2003).

The causative agent of septicemia, *Vibrio vulnificus* has been considered an important pathogen in humans due to its rapid pathogenic progresses and its high mortality rates (Hollis et al., 1976). This pathogen has been shown to exhibit an ability to form a biofilm (Joseph and Wright. 2004.). In the present study, we observed the processes for biofilm formation using the light and confocal microscopy. *V. vulnificus* showed distinct stages during biofilm formation, as shown in many bacterial species. The effects of growth conditions and genetic backgrounds on biofilm formation were investigated. Among the various parameters given to growth condition, the carbohydrate added as sole carbon source showed great effect. Those carbon sources resulted in increased production of exopolysaccharide (EPS), when determined by SDS-PAGE analysis of EPS extracts.

Screening of about 10,000 transposon-mutants revealed that the strains showing decreased capability of biofilm formation were mutated at the genes coding for transcriptional regulators. A quorum-sensing regulator, NtrC-homolog, is one of the regulators obtained in this assay, which is a well-known response regulator, and thus we examined whether NtrC-homolog played a role in biofilm formation by regulating the expression of several genes involved in biofilm formation. Since the mutant showed decreased production of EPS, the genes encoding enzymes involved in EPS biosynthesis were screened by *in silico* analysis, and the corresponding open reading frames were amplified and used for constructing transcriptional fusions and the specific null mutants. The results showed that the expression of each promoter was positively regulated by the functional NtrC-homolog, and the null mutants were decreased in both production of EPS and formation of biofilm. Therefore, it is proposed that a novel function of quorum-sensing regulator is involved in the critical steps in biofilm development of *V. vulnificus* by regulating EPS biosynthesis.

References

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