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Functional Switching of Serratia marcescens Transcriptional Regulator from Activator to Inhibitor of Quorum Sensing by Exogenous Addition

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Abstract : Some gram-negative bacteria enable the simultaneous activation of gene expression involved in N-acylhomoserine lactone (AHL) dependent cell-to-cell communication system. Such regulatory system for the bacterial group behavior is termed as quorum sensing (QS) because a diffusible AHL signal can accumulate around the cell during the increase of the cell density and trigger activation of the sequential QS process. By blocking the QS, the expression of diverse genes related to infection, antibiotic production, and biofilm formation is inhibited. Conditioning of QS by regulation of the DNA-receptor-AHL interaction is a potential target for enhancing host defenses against pathogenicity. We focused on engineered application of transcriptional regulator SpnR produced in opportunistic human pathogen Serratia marcescens. The SpnR can interact with AHL signals at an N-terminal domain and also with a promoter region of a QS target gene at a C-terminal domain. As the initial process of the QS activation, the SpnR forms a complex with the AHL to enhance the expression of pig cluster; the SpnR normally acts as an activator for the expression of the QS-dependent gene. In this research, we attempt to artificially control QS by changing the role of SpnR. The QS-dependent prodigiosin production is expected to inhibit by externally added SpnR in the culture broth of AS-1 strain because the AHL concentration was kept below the threshold by AHL-SpnR complex formation. Maltose-binding protein (MBP)-tagged SpnR (MBP-SpnR) was overexpressed in Escherichia coli and purified using an affinity chromatography equipped with an amylose resin column. The specific interaction between AHL and MBP-SpnR was demonstrated by quartz crystal microbalance (QCM) sensor. AHL with amino end-group was coupled with COOH-terminated self-assembled monolayer prepared on a gold electrode of 27-MHz quartz crystal sensor using water-soluble carbodiimide. After the injection of MBP-SpnR into a cup-type sensor cell filled with the buffer solution, time course of resonant frequency change (ΔFs) was determined. A decrease of ΔFs clearly showed the uptake of MBP-SpnR onto the AHL-immobilized electrode. Furthermore, no binding affinity was observed after the heat-inactivation of MBP-SpnR at 80ºC. These results suggest that MBP-SpnR possesses a specific affinity for AHL. MBP-SpnR was added to the culture medium as an AHL trap to study inhibitory effects on intracellularly accumulated prodigiosin. With approximately 2 μM MBP-SpnR, the amount of prodigiosin induced was half that of the control without any additives. In conclusion, the function of SpnR could be switched by adding it to the cell culture. Exogenously added MBP-SpnR possesses high affinity for AHL derived from cells and acts as an inhibitor of AHL-mediated QS.

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