

Quorum-Sensing in *Escherichia coli*: Discovery of A New Signal and Interfering with Cross-Talk via Furanones from Seaweed and Other Plants

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Abstract: In this study, DNA microarrays were used to determine the effect of stationary-phase signals on the gene expression of early exponential-phase *Escherichia coli* DH5 α cells that lack autoinducer-2 (AI-2). Fourteen genes were induced by fresh supernatants from a stationary culture, and 6 genes were repressed suggesting the involvement of indole and phosphate. Three genes were induced by autoclaved stationary-phase supernatant and 34 genes were repressed. In total, 3 genes (*ompC*, *ptsA*, and *btuB*) were induced and 5 genes (*nupC*, *phoB*, *phoU*, *argT*, and *ompF*) were repressed by both fresh and autoclaved stationary-phase supernatants. Furthermore, supernatant from *E. coli* DH5 α stationary culture was found to repress *E. coli* K12 AI-2 concentrations by 4.8-fold, suggesting the existence of an additional quorum sensing system in *E. coli* and that gene expression is controlled as a network with different signals working at different growth stages. Along with understanding *E. coli* signaling, we have also been interested in inhibiting biofilms with plant-derived compounds that do not affect bacterial growth rates; hence, there is no selection pressure against them. The quorum sensing disrupter (5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5H)-furanone (furanone) of the alga *Delisea pulchra* was shown by us to inhibit quorum sensing in *E. coli* via AI-2 (*Environ. Microbiol.* 3: 731, 2001). To study this further, DNA microarrays were used to investigate the genetic basis of this natural furanone inhibition of AI-2 signaling (statistically-significant values with $p < 0.05$ are reported). Using DNA microarrays, the AI-2 mutant *E. coli* DH5 α was compared with the AI-2 wild-type strain, *E. coli* K12, to determine how AI-2 influences gene expression. Then, *E. coli* K12 was grown with 0 and 60 $\mu\text{g}/\text{mL}$ furanone to study the inhibition of quorum sensing gene expression by furanone. It was found that 166 genes were differentially expressed by AI-2 (67 were induced and 99 were repressed) and 90 genes were differentially expressed by furanone (34 were induced and 56 were repressed). Interestingly, 79% (44 out of 56) of the genes repressed by furanone were induced by AI-2, which indicates the furanone inhibits AI-2 signaling and influences the same suite of genes as a regulon. Most of these genes have functions of chemotaxis, motility, and flagellar synthesis. Furthermore, the *E. coli* air-liquid interface biofilm formation was repressed by furanone, supporting the results that taxis and flagellar genes were repressed by furanone. The autoinducer bioassay indicates that 100 $\mu\text{g}/\text{mL}$ furanone decreases the extracellular concentration of AI-2 2-fold yet *luxS* and *pfs* transcription was not significantly altered; hence, furanone appears to alter AI-2 signaling post-transcriptionally. We will also show our work with other plant-derived biofilm inhibitors and preliminary results regarding the genetic basis of *E. coli* biofilm formation.