427q Ylih and Ycep Regulate *Escherichia Coli* K12 Biofilm Formation through Ai-2 and Indole

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ABSTRACT We have previously discovered that genes *yliH* and *yceP* are induced in biofilms 3- to 5-fold (*Appl. Microbiol. Biotechnol.* 64: 515-524, 2004), both are induced 12- to 28-fold compared to exponentially-growing suspension cells (*Mol. Microbiol.* 48: 253-267, 2003), and *yceP* was also found to have differential expression with respect to stationary phase cells (*Mol. Microbiol.* 51: 659-674, 2004, *Mol. Microbiol.* 48: 253-267, 2003). Here it is shown that deletion of *yceP* increases biofilm formation in microtitre plates by 3.5-fold in Luria-Bertani medium supplemented with 0.2% glucose (LB glu) and 2-fold in M9 minimal media supplemented with 0.4% casamino acids and 0.4% glucose (M9C glu). In continuous flow chambers with M9C glu using cells tagged with the green fluorescent protein and confocal microscopy, we found biofilm mass increased 240-fold, surface coverage 16-fold, and mean thickness 2762-fold relative to wild-type cells and that the roughness coefficient was observed to decrease 3-fold. Similarly, deletion of *yliH* was found to increase biofilm formation 2.4-fold in M9C glu in microtitre plates, and biomass increased 289-fold, surface coverage 31-fold, and mean thickness increased 2671-fold for cells in flow chambers with M9C glu. In addition the roughness coefficient was observed to decrease 8-fold in *yliH* relative to wild type. It was also found that deletion of *yliH* and *yceP* dramatically increased the motility of these two strains (2- and 6-fold, respectively). The change in motility occurred due to an increased transcription of several of the flagella and motility genes as confirmed through promoter transcriptional assays for *flhD*, *fliA*, *fliC*, *motA*, and *qseB* and as confirmed with the DNA microarrays. In addition, YliH and YceP were found to regulate biofilm formation through quorum sensing, and are believed to be involved in the internalization and export of AI-2 through catabolic repression. The deletion of *yliH* and *yceP* were found to increase extracellular AI-2 concentrations when grown in LB medium (most notably in the stationary phase) and this led to internalization of AI-2 when grown in LB glu (most likely through a cAMP dependent process). The DNA microarrays were used to study the gene expression profile for both the mutants relative to the wild-type strain in biofilms, and it was found 120 genes were induced and 14 were repressed consistently more the 3-fold ($p \leq 0.05$) in the *yceP* mutant, whereas 375 genes were induced and 159 were repressed consistently more than 3-fold ($p \leq 0.05$) in the *yliH* mutant. A surprisingly large amount of differentially expressed genes were found to be related to stress response and quorum sensing. In addition our microarray data showed that these mutants are likely to be involved in indole transport mechanisms; indole acts as an extracellular signal in *E. coli* (*J. Bacteriol.* 183:4210-4216, 2001) and as a regulator of biofilm formation (*Can. J. Microbiol.* 49: 443-449, 2003). Genes *acrE* and *acrF* involved in indole export were found to be unregulated 18- to 20-fold in our mutants, and *mtr*, known to be involved in the uptake of extracellular indole, was found to be down-regulated 2.5-fold in both of our mutants. Consistent with this data, genes *actD* and *gabT*, which are known to be activated by indole, were both repressed in both of our mutants probably due to decreased intracellular indole concentrations.