451g Analysis of Biofilm Architecture in *Escherichia Coli* Strains

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To relate differences in motility to their effect on biofilm architecture, five *Escherichia coli* strains, K12 M1655, K12 ATCC 25404, K12 BW25113, JM109, and DH5□, were studied in a M9 minimal medium containing 0.4 wt % glucose and casamino acids with a continuous flow system by using confocal microscopy and the green fluorescence protein (GFP). The biofilm structures of each strain were quantified using image analysis software COMSTAT. Wild-type strains K12 MG1655 and K12 ATCC25404 displayed scattered, branched macro colonies, and the COMSTAT parameters thickness, substratum coverage, and roughness coefficient corroborate the similarities between them (42 ± 14 □m vs. 44 ± 6 □m, 21 ± 8 % vs. 34 ± 13 %, and 0.51 ± 0.12 vs. 0.20 ± 0.09, respectively). *E. coli* JM109 displayed also scattered macrolonies but unlike K12 MG1655 and K12 ATCC25404, the macrocolony was smoother which was corroborated by the higher roughness coefficient (1.22 ± 0.13 for *E. coli* JM109). BW25113 was not able to develop a robust biofilm in this medium, and DH5□ appeared similar to JM109. These differences appear to be related to cell motility. Between the four strains, K12 MG1655 and K12 ATCC25404 displayed the highest motility (K12 MG1655 is slightly more motile than K12 ATCC25404) as *E. coli* K12 MG1655 displayed a 6- and 8-fold increase in motility compared to *E. coli* JM109 and *E. coli* BW251123, respectively. The genetic basis of the differences in motility were investigated and found to be due to differences in expression of the motility loci *qseB*, *flhD*, *fliA*, *fliC*, and *motA*. For example, K12 MG1655 has significantly higher expression of the motility quorum sensing locus *qseB* compared with K12 BW25113 (139-fold) and compared to JM109 (209-fold) that led to higher expression of *flhD*, *fliA*, and *motA* (which are controlled by QseB). These differences in the motility phenotype serve to explain the differences in biomass and architecture between each strain. Considering biomass, the fact that BW25113 was basically non-motile and displayed low expression of the motility-related genes indicates it is unable to form a mature biofilm in a continous system since it cannot move well and establish new colonies. Additionally, the effect of motility on architecture could be elucidated based n the differences on motility between both *E. coli* MG1655, *E. coli* K12 ATCC25404, and *E. coli* JM109 as JM109, which is the least motile of these three, displayed flatter macrocolonies compared with K12 MG1655 and K12 ATCC25404, which had more dramatic vertical structures as a result of its enhanced motility.