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Visualization and Characterization of the Pseudomonas aeruginosa Biofilm Matrix with Fluorescent Staining

The purpose of this study is to obtain 3-dimensional images of *Pseudomonas aeruginosa* biofilms, using fluorescent probes and confocal scanning laser microscopy (CSLM). The extracellular matrix material, which is primarily composed of polysaccharides, is very difficult to image because there are no universal stains for polysaccharides, and the structures of polysaccharides vary for individual strains of bacteria. In this study, we will use new fluorescent stains obtained from Molecular Probes to visualize both the Biofilm cells and their extracellular matrix material. This will allow us to obtain more complete 3-D images of microbial biofilm structures. Our hypothesis is that the cells stick to the matrix rather than the surface. We have chosen *Pseudomonas aeruginosa* as our test organism because it has the ability to produce three different polysaccharides, however each strain primarily only produces one of them. *P. aeruginosa* secretes these polysaccharides in its matrix in order to adapt to the environment. In our experiments, biofilms are cultured in a flow-through reactor. They are forced to form biofilms on glass coupons. At the end of day one, two, and three, biofilms are stained with six different dyes and epifluorescent microscope pictures are taken of both the cells and of the matrix.