**Non-Invasive Quantification of Plasmid Dynamics:**
**Biofilm Formation Effect on Plasmid conjugational plasmid transfer**

Hongyan Ma[1], James.D. Bryers[2]
[1] Department of Chemical Engineering, [2] Department of Bioengineering, University of Washington, Seattle, WA 98195-1720, USA

**Statement of Purpose:** The rates and efficiencies of TOL (pWWO) plasmid transfer in biofilms were evaluated under different environmental conditions, including different nutrient concentrations and externally antibiotic selection pressure at sub-lethal dosage. A multiple-labeling technique based on two GFP mutants combined with confocal laser scanning microscopy (CLSM) allowed the direct non-invasive quantification of the occurrence and growth of transconjugants. In this study, the spatial architecture of the heterogeneous biofilms was quantified to determine the contribution of nutrient concentration and sub-inhibitory concentration of an aminoglycoside antibiotic on plasmid transfer. In mature biofilms, low nutrient concentrations resulted in significantly porous biofilms, with open channels that enable more-frequent cell collision and deeper penetration of donor cells throughout the biofilms, thus leading to a more rapid spread of genes by conjugative plasmid transfer. In contrast, thick uniform, densely packed biofilms were formed under the condition of high nutrient concentrations, in which transconjugants were observed only at the outer layers of biofilm with lower plasmid transfer rates and efficiencies. Sublethal doses of kanamycin did not measurably alter the growth rate of bacterial populations in planktonic cultures. However, presence a sub-lethal dose antibitotic selection pressure significantly induced biofilm formation and substantially increased the efficiencies of TOL plasmid transfer in mature biofilms.

**Methods**: The donor bacterial strain of P. putida TUM-PP12 and plasmid TOL-gfpmut3b have been described in detail in Chapter 4.3.1. One strain that contained the DsRed reporter gene on its chromosome (TUM-PP10) and one strain that contained the GFP reporter gene the on TOL plasmid (AKN-PP11) were both used as bacterial control strains to vary and adjust microscopic settings. Pseudomonas putida KT2442 acted as recipient strain in all conjugation experiments. A continuous once-through parallel-plate flow cell system was used for biofilm formation [1]. The same LB broth and FAB chemically-defined media were also used to cultivate bacterial cells inoculums for flow cells. The supplementary carbon source of glucose with concentrations of 20, 50 and 200 mg/L and thiamine of 10 mg/L were used within the FAB chemically-defined medium for the biofilm formation within the flow cells. We describe a new approach, using confocal laser scanning microscopy combined with reporter-gene technology, in which the plasmid-bearing and plasmid-free cells number can be enumerated by detecting different fluorescent colors expressed by various reporter genes encoded on either plasmids or chromosome DNA.

**Results:** Biofilm can display a myriad of morphologies. Transfer frequency varied with depth in a biofilm. Plasmid transfer frequencies increase with increasing donor exposure time. Long donor exposure times enabled more frequent donor: recipient collisions, leading to faster plasmid conjugative transfer.

Despite the negative correlation between sublethal does of kanamycin and cell growth in planktonic cultures, the enhanced biofilm formation and higher efficiency of plasmid transfer were observed when biofilm populations were exposed to sub-inhibitory concentration of antibiotic.

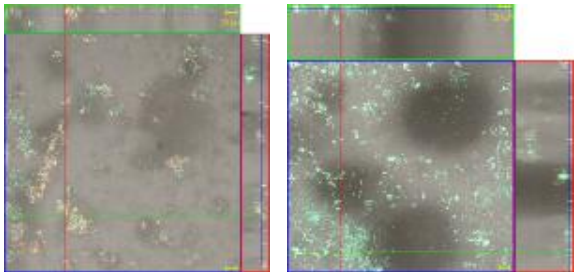

Figure 1&2. In situ monitoring of plasmid transfer in a biofilm cultivated in a continuous-flow cell system irrigated with FAB medium containing concentrations of 0 or 2.5 μg/ml Kanamycin, respectively.

**Conclusions:** Both the age of biofilms and the available nutrient concentrations play important roles in conjugational plasmid transfer in biofilms. At the early stage of pre-mature biofilms, nutrient concentration affects the formation of biofilms by varying the cell growth and biomass accumulation. Efficiencies of plasmid transfer appear to be independent of the variation in nutrient concentration in these early stages. In contrast, for mature biofilms with heterogeneous architecture (tulip-shaped clusters and water-filled channels and voids), the spatial structure of the biofilm has an impact on gene transfer. The availability of nutrients does not directly affect conjugational plasmid transfer within biofilms through metabolic effects on individual populations, but rather by indirectly affecting biofilm structure. Low nutrient concentrations resulted in porous biofilm clusters with open channels that enabled easier cell access and deeper penetration of donor cells throughout the biofilms, leading to faster spread of genes by conjugative plasmid transfer. Donors were found deep inside biofilms formed in flow cells. More donor cells in deeper biofilms were observed with longer incubation time between recipient biofilms and donor cells. In contrast, thick uniform and densely packed biofilm clusters were formed under the condition of high nutrient concentrations, where lower plasmid transfer rates and efficiencies were observed. Thus, penetration of donor cells within the biofilm is architecture dependent.

1. **References:** H. Ma, J. D. Bryers. J Ind Microbiol Biotechnol. 2010; 37:1081-1089.