Non-invasive Determination of Conjugative Transfer of Plasmids Bearing Antibiotic Resistance Genes in Biofilm-bound Bacteria: effects of substrate loading and antibiotic selection

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All CLSM images of the mature biofilms containing water channels and "tower-like" protrusions were analyzed by using Zeiss Examination software (LSM, Carl Zeiss, Jena, Germany) and "Image J" version 1.38v software (Research Services Branch, National Institute of Mental Health, Bethesda, Maryland; http://rsb.info.nih.gov/ij/). Images stacks comprised of different regions of transconjugants, recipients and donors were manually drawn around the edge of the outer viable zone using the "curve drawing" mode of the Zeiss Examination software (**Fig. S1 A**). Images at different z-sections within the same biofilm were all analyzed by this geometric mapping model. Structures of the biofilms were separated into four areas (**Fig. S1 A**), including the inner region of water-filled void (inner region within red boundary), donors (region between red and blue color boundaries), transconjugants (region between blue color boundaries) and recipients (region between white and outer blue color boundaries).

Two regions of the whole microcolony and recipients were chosen to present the correlations of the geometric parameters used in this viable mapping model. Similarly, the model was also applied to other regions for estimating these geometric parameters, which were used to discriminate differences of plasmid transfer events between the spatial structures of heterogeneous and uniform biofilms. The correlations based on structural geometry can be described by the following equations (**Fig. S1 B**):

$$R = \sqrt{\frac{A_1}{\pi}} \quad \text{and} \quad r = \sqrt{\frac{A_2}{\pi}} \tag{1}$$

$$L = R - r \tag{2}$$

$$\theta = \arctan\left(\frac{r}{h}\right) \tag{3}$$

$$L' = \left(\sqrt{\frac{A_1}{\pi}} - \sqrt{\frac{A_2}{\pi}}\right) \bullet cos\theta \tag{4}$$

$$V_{A,e} = \sum_{i=0}^{e-1}\left(h_{(i+1)} - h_i\right) \text{x } A_i \tag{5}$$

Where A_1 and R represent the total area and radius of a rounded microcolony, A_2 and r are the area and radius of the inner water voids of that same microcolony. L (in *x-y* plane) and L' (perpendicular to the bulk medium flow) are the averaged thickness of the recipients region. θ is the angle of the tower away from the normal (°), $V_{A,e}$ is the total volume of transconjugants or recipients, where A_i is the total area covered by cells of interest at position *i* in *x-y* plane, h_i is the distance from the substratum at position *i*.

At each section in the x-y plane, the areas of the total microcolony (A_1) and inner void (A_2) were measured by software directly. The angle θ of the tower can be calculated based on radius r and height *h* measured by the "arrow measure" mode of the Zeiss Examination software. First, one plots the radiuses (r) of the whole tower at different cross-sections versus the corresponding height h. Then, a linear trend line is constructed through the r vs. h plot, from which the slope r/h is determined. θ can then be solved from Eq. 3. The *"angle of cone away from the normal"* (θ), reflects the heterogeneity of the biofilm and the rigid properties of the microcolonies in mature biofilms. The "layer thickness at angle θ" (L´) measures the actual thickness between the layers of a microcolony and the bulk medium (L´ is perpendicular to the bulk medium flow). Clear differences on θ and L´ were observed between mature biofilms formed under the different nutrient concentration of glucose. It was found in this study that, with lower glucose concentrations, the values of θ and L´(R or D) were decreased but the total value of L´ (T) and the plasmid transfer efficiency was increased. The decreased thickness of recipients L´(R) and donors L´(D) were due to the metabolic activities of cells under the lower nutrient concentrations of glucose since additional growth of the populations after the events of transfer was involved in this experiment. The total decreased thickness of transconjugants L´(T) layer under the lower nutrient concentration is, at least in part, due to the combination effects of plasmid transfer and growth of transconjugants.

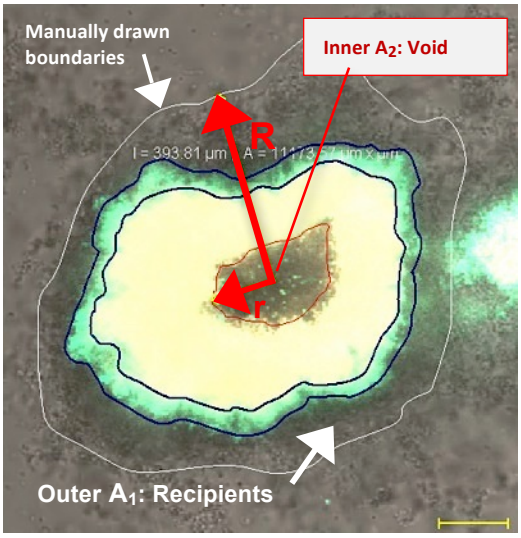

Figure S1 A. Manual selection of the regions: void (inner region within **red** line), donors (region between **red** and **blue** lines), transconjugants (region between two **blue** lines) and recipients (region between **white** and outer **blue** line). *X-Y* image collected from CLSM stacks at the *Z* position of 25 μm. Bar size = 20 μm.

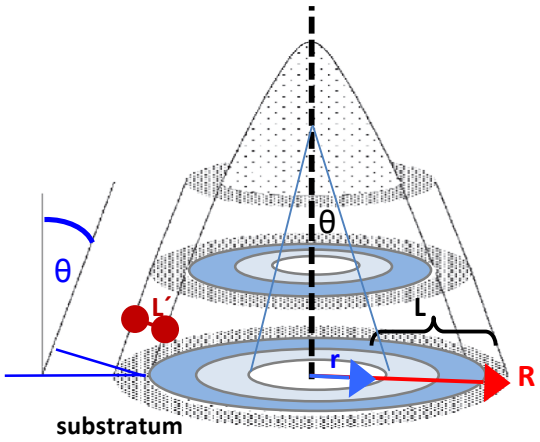

Figure S1 B. Geometric representation of a mature biofilm tower showing the void region (inner region), recipients region (outer), and the angle of the cone away from the normal (θ).

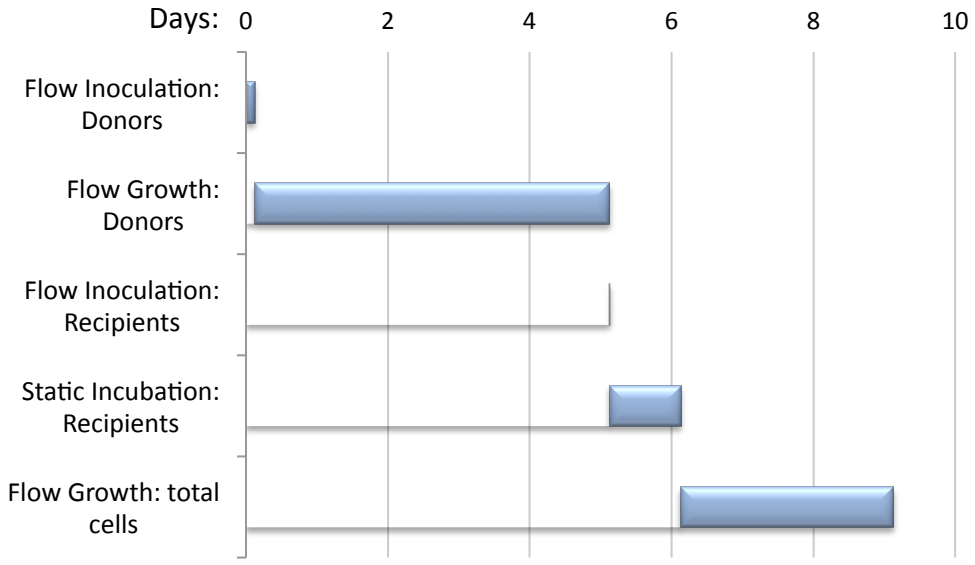

Figure S1 C. Gantt chart to present the sequence and duration of cell populations introduced to a continuous-flow cell system to monitor the plasmid under the presence of 200 or 20 μg/ml concentrations of glucose, respectively. Ttransconjugants (*P. putida* KT2442 with TOL-gfpmut3b plasmid), donors (*P. putida* TUM-PP12 with TOL-gfpmut3b plasmid), recipients (*P. putida* KT2442), and segregants (*P. putida* TUM-PP12 (miniTn5Putdsred)).

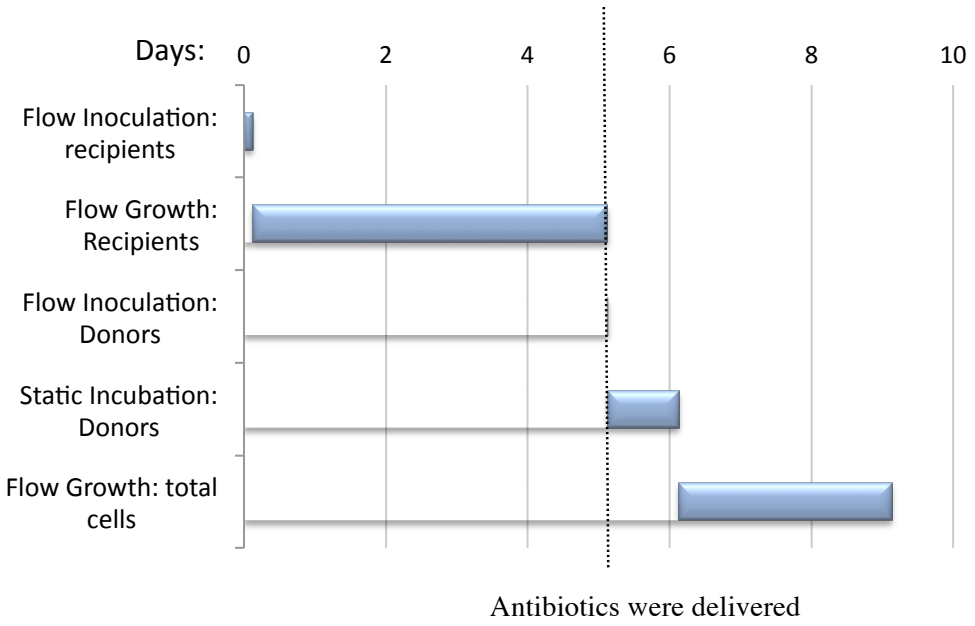

Figure S1 D. Gantt chart to present the sequence and duration of cell populations introduced to a continuous-flow cell system cultivated on FAB medium containing concentrations of 0 (Control, no antibiotics), 2.5 μg/ml Kanamycin or 0.1 μg/ml imipenem, respectively. Ttransconjugants (*P. putida* KT2442 with TOL-gfpmut3b plasmid), donors (*P. putida* TUM-PP12 with TOL-gfpmut3b plasmid), recipients (*P. putida* KT2442), and segregants (*P. putida* TUM-PP12 (miniTn5Putdsred)).