Non-Invasive Quantification of Plasmid Dynamics: Biofilm Formation Effect on Plasmid Segregational Loss

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Statement of Purpose: Plasmid DNA can provide a bacterial host an advantage in a selective environment. Under non-selective conditions in a natural environment, it is most likely possible that equilibrium exists between plasmid transfer, plasmid segregational loss and growth rates in mixed cultures [1]. Only a few studies have been carried out to examine the survival and loss of plasmids in natural habitats [2]. These studies, methods based on replica plating or PCR detection of genes inherent to the plasmids, suffer low sensitivity and under-estimation. In this study, the segregational stability, both in free suspension and developing biofilms without an applied selection pressure will be evaluated. Non-invasive microscopic analyses (Confocal Laser Scanning microscopy, CLSM) will be applied to quantify plasmid retention within developing pure culture bioflms. We hypothesizes that, compared with the expression of a heterologous gene in liquid culture, biofilm formation will affect the plasmid dynamics of stability and transfer and cloned gene expression in plasmid-bearing and plasmid free cells.

Methods: *P. putida* TUM-PP12 with TOL-gfpmut3b plasmid will serve as the host strain. This double-labeled strain derived from a strain of *Pseudomonas putida* (*Ppu*) KT2442 was chromosomally tagged with the DsRed gene and contains a GFPmut3b-modified plasmid TOL (pWWO) expressing the green fluorescent protein. LB Broth (10g/L) and FAB chemically defined medium [3] supplemented with glucose as the sole carbon source with the final concentrations of 2 g/L in suspended culture. Studies of biofilm formation was performance as described previously [3].A mass-balance-based quantitative mathematical model was developed by Huang ***et al.*** to evaluate the probability of segregational plasmid loss in both batch suspended versus biofilm growth cultures [17]. We describe a new approach, using a mathematical model 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: The average probability of plasmid loss in liquid suspension culture and biofilms in average calculated by this mathematical models are 0.0052± 0.0011and 0.016± 0.004, respectively. It was found that the probability of plasmid loss in biofilms cultures was statistical significantly greater than those in suspension cultures. Spatial distribution of plasmid-bearing and plasmid-free cells within the biofilms was determined using the "ortho" display mode of the CLSM. As seen in Figure 1, two populations of bacterial cells were detected as mushroom- or tulip- shaped microcolonies and clusters at different temperatures. A majority of cells expressing DsRed (plasmid-free or also called segregants cells) preferentially appeared on the top layers of existing microcolonies of the donor cells (yellow fluorescent) although a few segregants (cells lost pDNA) were also found deep inside biofilms. At 25ºC instead of 30 ºC, less segregants cells (red) were observed on the top layers of existing microcolonies of the donor cells. Figure 3 displays the distribution of probability of plasmid segregational loss at different biofilm depths with 25ºC and 30ºC performance conditions. At 30ºC, probability of plasmid segregational loss increased dramatically from 0.1% to 8% from the glass surface to the outside layers of biofilm. At condition of 25 ºC, probability of plasmid segregational follow the same trend but with lower absolute values.

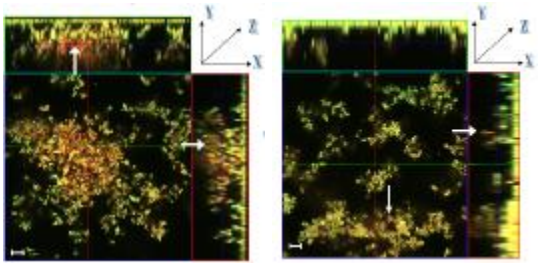

Fig 1&2 In situ monitoring of plasmid segregational loss in a biofilm studied by the use of CLSM at 25ºC and 30ºC.

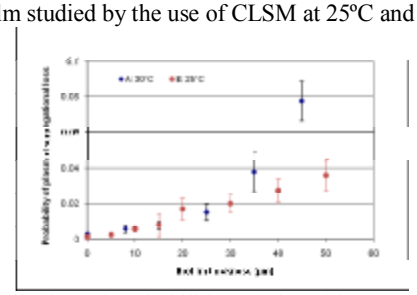

Fig 3 Distribution of Probabilities of plasmid segregational lost at the different depths of biofilm at 25ºC and 30ºC.

Conclusions: The probability of plasmid segregational loss within biofilms can be detected in situ directly. The overall probability of plasmid loss in biofilms cultures was greater than these inspected in suspended cultures significantly. However, biofilms are not uniform. Probabilities of plasmid segregational loss at different depths of biofilm changed dramatically. The highest probability of segregational loss occured at the outer layers of biofilms at 30 ºC since these is the most rapid growing cells. Cells adjacent to the substrate source are actively growing populations at the top layers of biofilms, while cells deep inside biofilms are frequently less metabolically active or dead due to substrate concentration gradients. Active cells have higher probability to loss plasmids, resulting in more plasmid-free cells. Nutrient limitation may thus partially be responsible for the distribution patterns of plasmid-bearing and plasmid-free cells within biofilm culture.

References:

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