Method for investigating the mode of action for biofilm preventing or biofilm disrupting wound products using gene expression analysis

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Introduction

The CDC Biofilm Reactor® is widely used for commercial product testing of biofilm established onto solid state coupons. Bacterial quantification through standard microbiological techniques is mainly used in the assessment of biofilm removal or prevention. However, it widely known that bacteria alter gene expression profiles as environmental pressures evolve. A greater understanding of key genes in response to the presence of antimicrobial products will allow wound companies to understand further about the mode of action of their products in relation to biofilm prevention and biofilm removal.

Aim

Identification of a novel method for screening antibiofilm products for their ability to prevent and disrupt biofilms, based on up-regulation and down-regulation of eight *Pseudomonas aeruginosa* biofilm genes.

Methodology

A CDC Biofilm Reactor® is a model that simultaneously houses biofilm encased bacteria and planktonic bacteria of the same culture strain. *Pseudomonas aeruginosa* was grown within a CDC Biofilm Reactor® containing stainless steel coupons for 72 hours such that both biofilm organisms and planktonic organisms could be recovered from the reactor.

Organisms were recovered at seven time points between 0 hours and 72 hours as the biofilm developed, and gene expression was monitored from both coupons and planktonic liquid cultures at the defined time points (Figure 1).

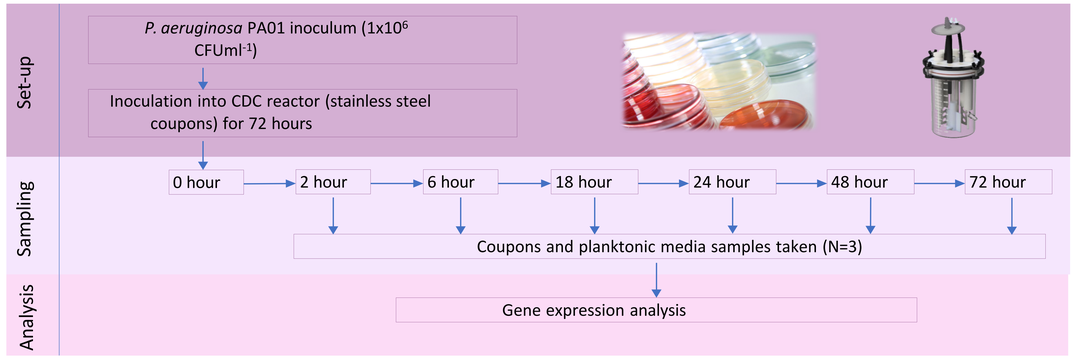

Figure 1. Methodology for the set-up, sampling and analysis of gene regulation during an up to 72 hour *Pseudomonas aeruginosa* growth in a CDC Biofilm Reactor® .

Results

- Genes in response to the transition from a planktonic to biofilm state (*rsaL*), type three secretion system function (*pcrV*), and nutrient utilisation (*cbrA*) were the most significantly upregulated (over 1,000x fold change)
- Significant expression of genes first seen at 6 hours (N=4) and 18 hours (N=4)
- Peak up-regulation occurred between 18 to 48 hours for all genes

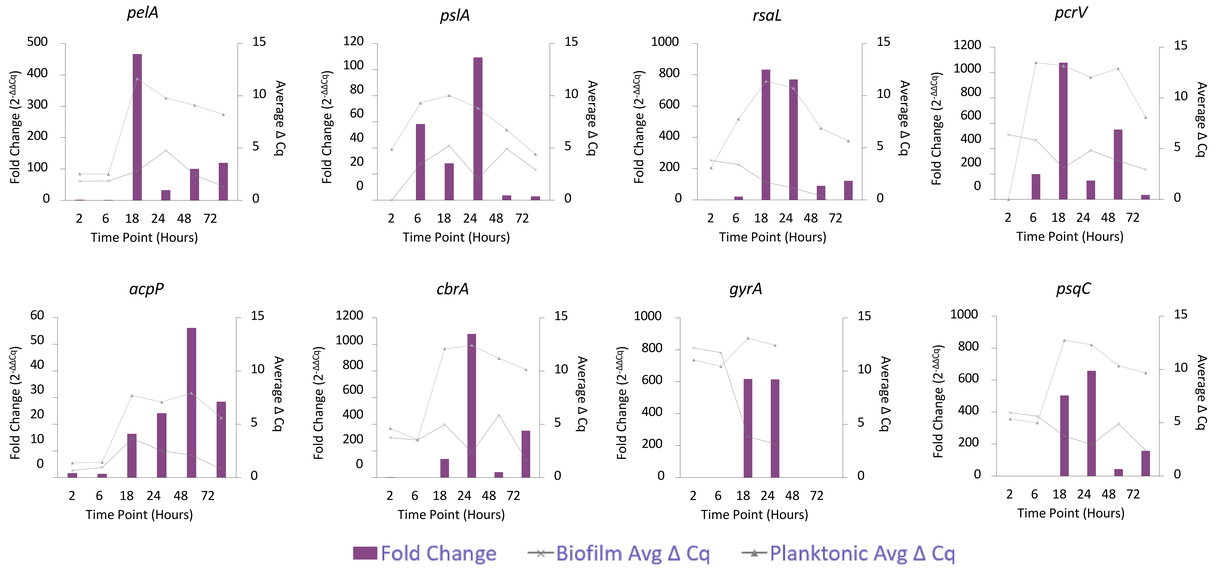

Figure 2. Individual gene transcription profiles for all eight target genes included in this study. Data is presented as the fold change difference in biofilm samples when compared to planktonic samples taken from the same time point.

Conclusions

- Standard growth conditions demonstrated peak expression for all eight genes between 18-48 hours post inoculation, this coincides with biofilm development that is typically reported to be early stage at 10 hours and to establish over 48 hours.
- Most genes (7/8) had over 100 times fold change in biofilm samples compared to planktonic demonstrating a clear opportunity to differentiate products that resulted in up or down regulation of these genes.
- This model provides a novel yet simple method for investigating product mode of action for biofilm preventing or biofilm disrupting products.

This project was carried out by Perfectus Biomed Group Now Part of NAMSA
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