P-139 - REVEALING THE DYNAMICS OF POLYMICROBIAL INFECTIONS: UPDATE ON THE QPCR AS A PROMISING TOOL FOR THE QUANTIFICATION OF BACTERIAL JUNGLES

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Abstract

P. aeruginosa and *S. aureus* are important opportunistic human pathogens in many polymicrobial infections. Interactions between them change the infection dynamics, resulting in increased potential for disease development. Determining the relative bacterial abundance through culture-dependent approaches is hindered by the presence of cells in a viable but poorly-cultivable state, or underestimated by the presence of cell-aggregates. The monitoring of bacterial pathogens by the use of culture-independent tools has led to fresh insights into the complex relationships between host and microbes, but often key experimental controls are lacking.
This study aimed at examining changes in microbial composition in *P. aeruginosa* (PA) and *S. aureus* (SA) communities by quantitative PCR (qPCR). Total RNA was extracted and normalized against the amount of an exogenous RNA control-molecule. The Cq value for each gene of interest was transformed into relative quantities taking the differences between the target and the calibrator exogenous mRNA.

Results & Conclusions

Despite its potential, several optimizations strategies had to be implemented in order to obtain reliable and meaningful quantifications of population dynamics by qPCR. Fluctuations in the Cq values for the *16S* rRNA from samples with the same amount of staring material highlight that the accuracy of mRNA quantification by qPCR is limited by mRNA losses during sample processing. Taking the mRNA losses detected, the gene expression of *PA* virulence-related genes was determined to assess if the inefficient mRNA recoveries could compromise the RNA functionality, providing an inaccurate transcription prolife. Results showed no significant differences in the gene expression of the selected genes.
Importantly, the use of the standardized exogenous mRNA was key to normalize mRNA losses across different samples. Without this exogenous control, the comparison between the expression level of the *16S* rRNA and the sample composition lead to misleading interpretations about the relative abundance of each species. Interestingly, we were able to demonstrate, as proof of concept, that using this normalization strategy, a good correlation ($P<0.05$) between the theoretical and the experimental PA/SA ratio was obtained.
Applying the qPCR-methodology coupled with the exogenous mRNA normalization strategy proved to be a reliable tool for the quantification of polymicrobial consortia.

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