Molecular Microbiology

P252

ANALYSIS OF GENE EXPRESSION VARIABILITY IN STAPHYLOCOCCUS EPIDERMIDIS BIOFILMS

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Staphylococcus epidermidis, a normal inhabitant of a human skin and mucosa, has emerged as one of the principle bacterial agents involved in nosocomial infections, particularly, in patients with indwelling medical devices. It's pathogenesis is related with the ability to adhere and form biofilms on the surface of those medical devices and is also associated with patients' immune system that can be compromised. This pathologic condition leads to a high morbidity and, uncommonly, mortality.

In the last decade, the quantification of gene expression has been one of the major areas of research in progress. The use of molecular biology techniques, such as quantitative (q) PCR, allowed the study of the process of biofilm formation, which is very complex and involves elaborated genetic regulation. When determining the quantification of genetic expression, there are two critical experimental steps that can impact the outcome of the experiment: RNA extraction, traditionally considered the crucial step of the whole experience, and reverse transcriptase reaction. We have previously shown that in *S. epidermidis* biofilms, RNA extraction procedure has a strong influence on the outcome of gene expression quantification. Here we evaluated the individual contributions of all experimental steps in the outcome of reliable gene expression determinations. To achieve that, we determined the expression of *aap*, *fmtC* and *lrgB* genes using the type strain RP62A, by performing technical duplicates of each experimental step, and evaluating thecoefficient of variability. Interestingly, our results showed that the bulk of the variability of the gene expression quantification derived from the biological replicates, and not from any of the experimental steps. Furthermore, variability from RNA extraction was not significantly different from the variability obtained from reverse transcriptase or qPCR experiments. This study further confirms that biofilms are difficult biological samples with enhanced difficulties in gene expression determinations.