

Transcriptional analysis in microbial fuel cells: common pitfalls in global gene expression studies of microbial biofilms

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The potential for microbial fuel cells to act as an alternative, pollution-neutral energy source has generated a major increase in the number of publications on this subject. Fundamental to the functioning of a microbial fuel cell, and the efficient transfer of electrons to an associated electrical network, is the formation of specialized biofilms on an electrode surface. Microarray studies of these biofilms have important considerations that are also fundamental for biofilm gene expression studies in general. Cells in a biofilm exist in a range of different physiological states, but global analysis generalizes transcription across the entire biofilm population. This leads to the common pitfall of a complex system being overly simplified.

Bacteria are commonly found in the environment as part of surface-associated communities known as biofilms. Through the formation of a biofilm, bacteria gain many advantages, such as an increased resistance to desiccation, resistance to antibiotics, defence against grazing, and increased metabolic function, among others. Biofilms are studied extensively due to their importance in environmental, industrial, and medical processes. They are highly hydrated structures containing cells encased in an extracellular matrix of proteins, DNA, enzymes, and extracellular polymeric substances. Many bacterial biofilms consist of structured clumps of cells surrounded by channels void of

cellular material, in which nutrients and waste can be exchanged, resulting in a diverse range of microenvironments. For example, electrical-producing biofilms in a microbial fuel cell can be > 50 µm thick, have been shown to contain proton gradients, and are suspected to contain electrical potential and nutrient gradients.

Transcriptional profiling has become the tool of choice for microbiologists to examine changes in gene expression. Microarrays are powerful tools that allow for the examination of genome-wide changes in gene expression in either isogenic mutant strains or different environmental conditions. The rapid analysis of genome-wide expression has accelerated our understanding of microbial responses to changing conditions. The global analysis of transcription within a bacterial biofilm is an appealing technique to identify genes and specialized gene expression patterns associated with biofilm formation, without the need for extensive and time-consuming studies of individual genes (Beloin & Ghigo, 2005; An & Parsek, 2007). The reduction in the cost of genome sequencing and the availability of custom microarrays has resulted in an increase in studies using microarrays to investigate gene expression in biofilms of their bacteria of interest. However, interpretation of results from these studies is problematic because RNA is extracted from cells throughout a biofilm, which are in a wide range of metabolic states.

To obtain enough biofilm material for transcriptional profiling, the entire biofilm is normally collected for RNA extraction. This is a major problem, because cells with a range of different physiological and phenotypic states are used for comparison against a homogeneous planktonic culture. Small differences between experimental setups can thus lead to large differences in results. This has been highlighted by the comparison of three independent microarray-based studies of the *Pseudomonas aeruginosa* quorum-sensing regulon (An & Parsek, 2007). The independent studies contained as many differences as similarities even when a fivefold change was used as threshold. While reproducibility may have been an early major concern for microarray studies, this issue highlights the importance for researchers to consider what is actually being compared. In microbial fuel cells, there are a number of processes that can occur within the biofilm. Put simply, expression of

individual genes may play a role in the process of biofilm formation, in the process of extracellular electron transfer, or in both. To understand these processes in a current-producing *Geobacter sulfurreducens* biofilm, microarrays have been used to compare gene expression in electrical biofilms, to both planktonic cells and nonelectrical biofilms. These microarrays were designed to examine genes important for biofilm formation and/or genes important for extracellular electron transfer in a biofilm. In these cases, many targets have been identified. However, their importance could only be confirmed through mutational analysis, which identified important features such as nanowire production and extracellular cytochromes for power production, and/or biofilm formation.

This highlights an important consideration: how are transcriptome data to be used? Typically, a quantitative reverse transcriptase-PCR reaction is used to corroborate the microarray results. Although useful, this process provides no spatial information about expression within the biofilm. This is a very challenging aspect of biofilm studies. A common technique for tracking microbial gene expression is using short half-life fluorescent proteins combined with confocal scanning laser microscopy. But this process is limited to systems that can be placed within the imaging distance of a confocal microscope, to bacteria with a genetic system allowing the use of such expression systems, and to systems with enough oxygen present to allow correct folding of the fluorescent protein with the short half-life. Also, the commonly used glass flow cells are highly artificial surfaces for microbial growth. It took considerable effort to construct a real-time imaging microbial fuel cell, which is currently limited to *G. sulfurreducens* due to its genetic amiability for fluorescent protein expression. The ability to examine the electricity-producing biofilm nondestructively has allowed for the direct measurement of proton accumulation and metabolic activity within the biofilm through the use of fluorescent dyes.

A recent development that is helping to overcome some of these inherent problems is a technique to spatially extract RNA from a biofilm in sufficient quantity and quality for microarray analysis from a single biofilm (Franks *et al.*, 2010). Using a single biofilm, a spatial examination of gene expression can be performed, providing valuable information for internal transcriptional differences within the biofilm. Because small differences between biofilms can

cause large differences in transcription, these differences can be minimized through the use of a single biofilm. Once again, the experimental design should consider that genes important throughout the biofilm might not be differentially expressed spatially, even though they are fundamental for function. However, gene transcription does not always indicate protein localization. Even though transcription is detected in a biofilm, translation and protein localization may not follow the same pattern, which requires further protein fusion and labelled antibody studies.

Microbial biofilms are extremely important, but the examination of gene expression is still fraught with pitfalls and overgeneralizations. Microarray analysis is a wonderful tool, especially as the costs are continually decreasing, making their use much more routine. However, it is essential to remember that they are only a starting point for biofilm research and not a tool that can be applied without careful consideration of experimental design and follow-up research. Without this caution, researchers may overinterpret results and assign them greater significance than deserved. Although large data sets of significantly up- and down-regulated gene expression patterns are created, often only a few genes with real phenotypic importance are identified in biofilm microarray studies.

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