# High variability of gene expression in *S. epidermidis* biofilm population

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In the past two decades *S. epidermidis* has emerged from a commensal microorganism into a predominant opportunistic pathogen associated with nosocomial infections due to the ability to adhere to abiotic surfaces and form biofilms. The increasing use of indwelling medical devices has influenced the rise of *S. epidermidis* to a major medical research topic. *S. epidermidis* biofilms are well known to be resistant to both the host immune response and antimicrobial therapy, making these infections hard to treat and often resulting in recurrent infections. To better understand why biofilms have evolved in this manner, many comparative studies have been performed using biofilms and planktonic cultures. However, since biofilm cultures are fundamentally different from planktonic cultures, some concerns have been raised in the past for such studies. While it seems reasonable to compare biofilm cultures with stationary planktonic cultures, recently it has been suggested that biofilms cultures could be compared directly to the bacteria in suspension, grown in the vicinity of the biofilms. Nevertheless and however interesting, this suggestion fails to accommodate the fact that mature biofilms will release bacteria from within the biofilm to the suspension. This phenomenon was suggested to be responsible for colonization of further niches. Therefore, such population can both contain biofilm outbound bacteria as well as plantkonic free floating bacteria.

In an attempt to better understand the possible differences between cell populations, we selected 5 distinct bacterial isolates previously characterized for biofilm formation and compared the expression of some genes of interest, namely *atlE* (involved in initial adhesion) and *icaA* (involved immune evasion and biofilm maturation). Three populations were characterized: (1) late exponential planktonic cultures grown on Erlenmeyer flasks, (2) biofilm populations attached to polystyrene 24-well culture plates and (3) the bacteria grown in suspension on the same well of culture plates as the biofilms (non-adherent cells), all grown in TSB supplemented with 0,4% glucose. Differences in the gene expression profile were observed between *S. epidermidis* strains. The *icaA* expression values were generally higher in biofilms as compared with planktonic cultures. However, when comparing with the non-adherent cells grown in the vicinity of the biofilms, some strain to strain variation was observed, as in some cases the non-adherent cells has lower *icaA* expression but in other instances the opposite occurred. A similar effect occurred with *atlE* expression. A possible explanation for the higher variation on the non-adherent cells has to do with the washing step required before resuspending the biofilm: while more tenacious biofilm forming strains will withstand better the washing step, some weaker biofilm forming strains will be washed away. In the latter cases the bacterial population described as non-adherent cells will be very heterogeneous. Thus, with the variation found in the non-adherent bacteria, it seems that in order to study the physiological differences that occur when bacteria are living in a biofilm, planktonic cultures grown independently of biofilms should be used to better understand the pathophysiology of the biofilm-related infections.

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