RELATIONSHIP BETWEEN GENES INVOLVED IN ANTIBIOTIC RESISTANCE AND BIOFILM PRODUCTION IN BIOFILM-FORMING BACTERIA

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ABSTRACT

The majority of bacteria in nature reside in complex sessile communities known as biofilms that are firmly attached to biotic or abiotic surfaces. The diverse microbial organisms present in biofilms as a compact group of microbial consortia show extraordinary resistance to conventional biocides, antibacterial treatments, and the immune defense responses of the host. The formation of these sessile communities and their intrinsic resistance to antibacterial treatments are at the root of many persistent and chronic bacterial infections. The increased tolerance of bacteria in biofilms towards antibacterial compounds and the host immune system constitutes a central issue in the treatment of bacterial infections. Studies proved that specific physiological growth conditions of biofilm and genetic interactions within the biofilms caused a dramatic increase in tolerance to the antibacterial agents. The expression of biofilm-related genes is highly correlated with phenotypic biofilm development. Therefore, this review focuses on the genes involved in antibiotic resistance, biofilm production and its expression. There are various methods available for analyzing gene expression in biofilm formation. Especially, microarray analyses *in vivo* expression technology has recently been used to study gene expression in biofilms. Recent genomic studies have identified many of the genes and gene products differentially expressed during biofilm formation, revealing the complexity of this developmental process of biofilm. It has been demonstrated that the levels of gene expression between biofilm and planktonic populations differ significantly. These differences could be the result of quorum-sensing mechanisms or the adaptability of bacteria to show increased tolerance to different stresses. However, a comparison of the differentially expressed gene sets identified in biofilm bacteria reveals that no common expression pattern for biofilms has been identified yet. The genes associated with biofilm formation are found to be up- and down-regulated differently in different scenarios. However, understanding the genetic and molecular basis of bacterial community behavior will lead to potential therapeutic targets that could consequently lead to reductions in mobility and mortality rates.

Key words- Biofilm, Biofilm forming genes, Antibiotic resistance

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INTRODUCTION

Biofilms have a well-defined architecture, consisting of tower- and mushroom-shaped microcolonies embedded in a sessile matrix of polymeric substances, polysaccharides and proteins that are produced by the resident microorganisms. The formation of these sessile communities and their inherent resistance to antibacterial agents are at the root of many persistent and chronic bacterial infections consequently leading to an increase in morbidity and mortality rates. Around 65–80% of all bacterial infections are related to biofilm formation. Biofilms have been shown to colonize a wide variety of medical devices and to be associated with several human diseases, such as native valve endocarditis, burn wound infections, chronic otitis media with effusion and cystic fibrosis. Compared with the planktonic bacteria, the compact microbial communities present in biofilms show strong resistance to antibiotics and the immune defense mechanisms of the host. Poor penetration of antibiotics through a biofilm to its depth is the general reason for the resistance. Moreover, the expression of biofilm-related genes is highly correlated with phenotypic biofilm development and genetic interactions within the biofilms caused a dramatic increase in tolerance to the antibacterial agents. Further, the existence of antibacterial resistance genes in biofilms not only provides resistance but is also involved in the development of biofilm (Sauer, 2003; Al-Bayati & Samarasinghe, 2022).

Antibiotic Resistance Genes in Biofilm Producing Bacteria

The resistant genes encoded in the chromosomes are transferred among bacteria in the biofilm through transformation, conjugation, or transduction which generate biochemical defense mechanisms against certain antibiotic compounds. The gene exchange allows the resistance in biofilm-forming bacteria which causes major problems for physicians treating infectious diseases (Vivehananthan *et al*., 2020). Strong biofilm formation was observed for both Carbapenem Resistant *Escherichia coli* and *Klebsiella pneumoniae*, which carry the bla resistance genes, compared to the non-resistant control strains. The rate of stronger biofilm formation was similarly observed in other studies of multidrug-resistant *Enterobacteriaceae* (Al-Bayati & Samarasinghe, 2022), Whereas, icaA and icaD genes involved in the formation of slime and biofilm found in *Staphylococcus aureus* and *Staphylococcus epidermidis* (Nourbakhsh & Namwar., 2016). Moreover, cupA, bssS, and fimH genes are responsible for surface adhesion and biofilm formation in *Pseudomonas aeruginosa*. Also, it has been shown that clpP is essential for biofilm formation in *Pseudomonas fluorescens* (Sauer, 2003). Though the resistance genes have been identified, there have been fewer studies so far to demonstrate the relationship between biofilm formation and related gene expression.

Genes Expression Pattern in Biofilm Formation

Bacteria employ numerous mechanisms to regulate the transition between an unattached form to a biofilm bacterium. One form of bacterial cell–to-cell communication is known as quorum sensing (QS). QS is a chemical signaling system that controls gene expression and regulates bacterial virulence, including bacterial motility, adhesion, and biofilm formation (Ruiz, 2019).

The expression of some resistance genes is induced by the antibiotics. DNA microarray studies reveal that no common expression pattern has been identified yet for the genes involved in biofilm

formation. Different genes have been noticed to be up-and down-regulated in multiple studies, in various levels ranging from 1% to 38% of the entire genome. The majority of the differentially expressed genes were associated with motility and chemotaxis. However, motility and chemotaxis appear to be associated with only the earliest stages of biofilm production, such as the transition from planktonic to sessile modes of growth (Sauer, 2003).

The gene expression in *E. coli* biofilms and planktonic cells was examined in a research study, and the results showed that the overall expression of only 79 genes, representing 1.84% of the *E. coli* genome, was remarkably altered during biofilm growth compared with planktonic growth. The genes slp and ompC have been associated with the initial steps of *E. coli* biofilm formation on abiotic surfaces and showed increased expression. In another DNA microarray analysis study of *Pseudomonas aeruginosa*, only 1% of the genes were found to be differently expressed in biofilm development mode, with 0.5% of the genes being activated and around 0.5% being repressed. Also, gene expression was analyzed in *Bacillus subtilis* using microarrays and a total of 519 genes were identified as differentially expressed during biofilm formation (Sauer, 2003).

Detection of Gene Expression Levels in Biofilm-Producing Bacteria

A number of microarray-based methods for the detection of differentially expressed genes in biofilm formation have been described in various studies. DNA microarrays of an organism are typically used to determine which genes are controlled by a particular transcription factor or environmental signal. Thus, DNA microarray studies are usually carried out as a comparison of two samples to identify differentially expressed genes (Lazazzera, 2005). In addition to microarray analyses, *in vivo* expression technology (IVET) has recently been used to study gene expression in biofilms. In IVET, the complex environment of interest is used to screen a reporter gene library and to select those clones in which gene expression is on (Finelli, 2003). Other than this biofilm gene expression analysis by quantitative real-time PCR (qPCR) has been increasingly used to understand the role of biofilm formation in the pathogenesis of several bacteria (Franca *et al*., 2012). Also, Restriction Fragment Differential Display PCR (RFDD-PCR) analysis is a sensitive and specific method to identify genes which are typically expressed in biofilms. Most of the other methods have the disadvantage that large quantities of mRNA are required. However, RFDD-PCR has the advantage that only minimal quantities of total RNA are needed (Zhengwei & Zheng., 2005).

CONCLUSIONS

Knowledge of the characteristics of biofilm formation, their antibiotic resistance patterns and gene expression would be helpful for therapeutic decisions and useful for antibiotic therapy in infected patients. Only a limited number of studies on the expression profiles of genes are involved in biofilm production. It is apparent that biofilms have gene-expression patterns that differ from those of planktonic bacteria. However, more studies are required to gain knowledge on a complete description of the genomic and physiological changes that occur during biofilm formation. Studies of the genomics behind biofilm formation would lead to the identification of novel treatment strategies. Identification of novel therapeutics would massively reduce the infection rate, thereby reducing morbidity and mortality rates in the future.

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