Biofilm-Related Diseases and Omics: Global Transcriptional Profiling of *Enterococcus faecium* Reveals Different Gene Expression Patterns in the Biofilm and Planktonic Cells

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Abstract

*Enterococcus faecium* is an opportunistic pathogen with a remarkable ability to acquire resistance toward multiple antibiotics, including those of last-resort drugs such as vancomycin and daptomycin. The occurrence of vancomycin-resistant *E. faecium* is on the rise and there is a need to understand the virulence of this organism. One of the factors that contributes to the virulence is the ability to form biofilms. Since bacteria in biofilm state are more resistant to antibiotics and host immune response, understanding the molecular mechanism of biofilm development is important to control biofilm-related diseases. The aim of this study was to determine the global gene expression profiles of an *E. faecium* strain, VREr5, during the early event of sessile growth compared with its planktonic phase through RNA-sequencing approach. The results clearly illustrated distinct expression profiles of the planktonic and biofilm cells. A total of 177 genes were overexpressed in the biofilm cells. Most of them encode for proteins involved in adherence, such as the *ebpABC* locus. Genes associated with plasmid replication, gene exchange, and protein synthesis were also upregulated during the early event of biofilm development. Furthermore, the transcriptome analysis also identified genes such as *fsrB*, *luxS*, and *spx* that might suppress biofilm formation in VREr5. The putative biofilm-related *bee* locus was found to be down-regulated. These new findings could provide caveats for future studies on the regulation and maintenance of biofilm and development of biomarkers for biofilm-related diseases.

Keywords: biofilm, differential gene expression, *Enterococcus faecium*, RNA-seq, transcriptomes

Biofilm is a surface-associated community of microorganisms encased in a matrix of exopolymeric substances. It has been shown that sessile/biofilm cells are generally more tolerant to antibiotics and host phagocytosis than their planktonic counterparts due to the multilayered structure of mature biofilm, slower metabolic rate, and the ease of gene exchange within the biofilm community (de la Fuente-Núñez et al., 2013; Donlan and Costerton, 2002). Hence, biofilm-associated infections are often difficult to eradicate. Many chronic diseases such as endocarditis, periodontitis, and otitis media are associated with bacterial biofilms (de la Fuente-Núñez et al., 2013). Moreover, a wide variety of medical devices such as catheters and prosthetic heart valves are prone to being colonized by biofilm-forming bacteria. Clinical *E. faecium* appeared to have higher occurrence of biofilm formation as compared with nonclinical isolates (Almohamad et al., 2014),
In comparison with the planktonic cells, biofilm cells are more resistant to environmental stresses. A global transcriptional regulator Spx has been shown to be involved in various stress responses, especially in oxidative stress (Kajfasz et al., 2012; Nakano et al., 2003). However, it appears that Spx did not participate in the general stress response of the biofilm formed by VREr5 as the gene encoding it was downregulated. The spa mutant strains of both *Staphylococcus aureus* and *Staphylococcus epidermidis* were reported to form biofilm more profoundly as compared with the wild type (Pamp et al., 2006; Wang et al., 2010). These results suggest that *spa* has a negative effect on the biofilm formation of these two studied species. In both cases, *spa* disrupts the expression of the *ica* operon encoding polysaccharide intracellular adhesin. This leads to the reduction of primary attachment and subsequently affects biofilm formation. Similarly, *spa* may also negatively control the expression of biofilm-related gene(s) in VREr5. Wang et al. (2010) demonstrated that the protease ClpP was involved in biofilm formation of *S. epidermidis* by degrading Spx. However, our results showed that clpP was underepressed in the biofilm cells. This implied that clpP may not be involved in the *spa*-mediated biofilm formation of VREr5. Alternatively, the downregulation of clpP may be due to the presence of an autoregulatory system, which is activated when the level of substrate (Spx) is below the threshold.

In contrast to other transcriptome studies (Beenken et al., 2004; Resch et al., 2005), we did not observe a higher level of expression of genes encoding the arginine deiminase system (*arcABC*) in the biofilm cells. Instead, these genes were among the highly downregulated genes in the biofilm formed by VREr5. The arginine deiminase system is involved in the pH homeostasis in biofilm (Lindgren et al., 2014). During the maturation of biofilm, microaerobic conditions developed in some regions due to the enclosed matrix. In these regions, acid production increases as a result of fermentation, creating acid stress. The *arcABC* operon converts arginine to ornithine, ammonia, and carbon dioxide (Cunin et al., 1986). The ammonia is then protonated into ammonium ion (\(\text{NH}_4^+\)), which increases the intracellular pH, compensating the acid stress (Fulde et al., 2011). Apart from that, the pathway also generates ATP, which serves as an alternative energy source when sugars are depleted during the course of biofilm development. Conversely, in the initial stages of biofilm formation, nutrients and oxygen are still sufficient for energy production through aerobic respiration, and a microaerobic environment that might contribute to acid stress has not been built up. Hence, the *arcABC* operon plays a more important role in a mature biofilm, as demonstrated by Lindgren et al. (2014) and Resch et al. (2005). The underexpression of *arcABC* observed in VREr5 might be related to its minor role in the initial stage of biofilm formation. However, given the large fold change observed between the planktonic and the sessile cells, the actual role of *arcABC* in biofilm formation of VREr5 warrants further investigation.

Our previous whole genome sequence analyses identified a homolog of *E. faecalis* **bee** locus in VREr5, which might be associated with biofilm formation (Lim et al., 2017). The **bee** locus consists of five genes (*bee-1*, *bee-2*, *bee-3*, *srp-1*, *srp-2*), which encode for proteins putatively involved in ligand binding and cell wall anchoring (Tendolkar et al., 2006). Inactivation of **bee-2** results in 70% reduction in the biofilm-forming ability of *E. faecalis*. Furthermore, Tendolkar et al. (2006) also demonstrated enhanced biofilm formation in the **bee** locus transconjugants compared with the parent strains, confirming the role of this locus in biofilm development of *E. faecalis*. Unexpectedly, our transcriptomic analysis showed that the **bee** homolog was underepressed in the biofilm cells relative to that of the planktonic cells. In our previous study, VREr5 was the only biofilm former among the four studied strains and was the only strain that harbored the **bee** homolog (Lim et al., 2017). The gene expression data obtained in this study suggested that the locus might play no or minor role in the biofilm growth of VREr5. Whether or not **bee** locus contributes to other functional roles in VREr5 is yet to be determined.

Interestingly, many genes that are known to participate in biofilm formation of *E. faecium* were not differentially expressed. These included the *esp*, *acr*, and *sgrA*. Although many studies had documented the association of *esp* to biofilm formation in enterococci, other studies suggested that *esp* is not required or sufficient for biofilm formation in *E. faecalis* and *E. faecium* (Dworniczek et al., 2005; Ramadhan and Hegedus, 2005). These inconsistent results imply that *esp*-mediated biofilm formation is possibly strain specific. The exact mechanism of *esp*-mediated biofilm formation is still under active investigation (Wang et al., 2010). Both genes encode for substrate-binding proteins that have been implicated in biofilm formation in *E. faecium* (Hendricks et al., 2009; Nallapareddy et al., 2008). The lack of induction of these genes under the studied condition suggested that the positive effect of *acr* and *sgrA* on *E. faecium* biofilm is probably strain specific, similar to *esp*. Alternatively, these genes might only be needed at the later phase of biofilm development. Hence, the induction of these genes can only be observed if the biofilm was allowed to grow for a longer period of time. The latter postulation was supported by the underexpression of *algE*, and *sagA*, which encodes for an autolysin and secreted protein, respectively, which have been shown to participate in the maturation of biofilm (Pagnelli et al., 2013, 2015).

### Conclusions

Using RNA-seq approach, we presented the gene expression profile of a vancomycin-resistant *E. faecium* strain at the initial stage of biofilm formation. One hundred and seventy-seven genes have been significantly upregulated in the biofilm cells. These included genes that are involved in adherence, plasmid replication, horizontal gene transfer, and protein synthesis. The induction of these genes can only be observed if the biofilm was allowed to grow for a longer period of time. The latter postulation was supported by the underexpression of *algE*, and *sagA*, which encodes for an autolysin and secreted protein, respectively, which have been shown to participate in the maturation of biofilm (Pagnelli et al., 2013, 2015).

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