The influence of Enterococcus faecalis on biofilm development and antimicrobial susceptibility of Streptococcus mutans


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Introduction

Dental plaque or oral biofilm was first described by Van Leeuwenhoek in the 17th century when he already sketched the reality of very complex dental biofilms. The role of micro-organisms in tooth decay was first described by Miller in 1890 and since then oral microbiology has come a long way. Understanding bacterial interactions and their response to antimicrobial treatment is still a common research topic and in vitro tests with single species biofilms of non-clinical lab strains is still being performed. Unfortunately these antimicrobial susceptibility tests rarely extrapolate to the in vivo reality because of the variability in species that make up the biofilms in the oral cavity. In previous studies we have found that co-culturing Enterococcus faecalis strains with a Streptococcus mutans strain (Deng et al., 2009), both from clinical origin, results in a variability in antimicrobial resistance which is dependent on the E. faecalis strain used. To comprehend this phenomenon we have co-cultured several clinical S. mutans strains with one E. faecalis strain and assessed their biofilm-forming properties and antimicrobial susceptibility to a chlorhexidine treatment.

Method

Twenty-four hour S. mutans biofilms of 7 different strains were grown in a Transferable Solid Phase (TSP) system, followed by the introduction of E. faecalis and an additional incubation for 24 hours. The influence of E. faecalis on S. mutans biofilm development was determined by crystal violet biomass staining. To assess the antimicrobial susceptibility of single and dual species biofilm, a resazurin metabolism assay was performed.

Results

As shown in figure 1, the biomass staining revealed that the presence of E. faecalis OS16, significantly (p<0.05) decreased the biofilm biomass in dual species biofilm when compared to single species S. mutans biofilm. This reduction was strain dependent and varied by 30-97% from strain to strain.

The data from the resazurin assay, see figure 2, showed that the presence of E. faecalis in a dual species biofilm resulted in a statistically significant increase in resistance to a CHX treatment (p<0.05) for all S. mutans strains, except for C180-2. This increase ranged from 12-51% of remaining metabolic activity when compared to 2-13% in single species S. mutans biofilm.

Conclusion

The presence of E. faecalis influences biofilm development of S. mutans in a dual species biofilm and enhances antimicrobial resistance towards a sub-lethal chlorhexidine treatment in an S. mutans strain dependent way. These findings support our idea, that antimicrobial susceptibility testing should be performed on a range of clinical isolates and preferably in a polymicrobial setting.

References

Van Leeuwenhoek, Letters to the Royal Society in London, September 17, 1683.

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