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

M.A. Hoogenkamp*, D.M. Deng, S.K. Bierman, W.Crielaard

Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and VU University Amsterdam, Department of Preventive Dentistry

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.

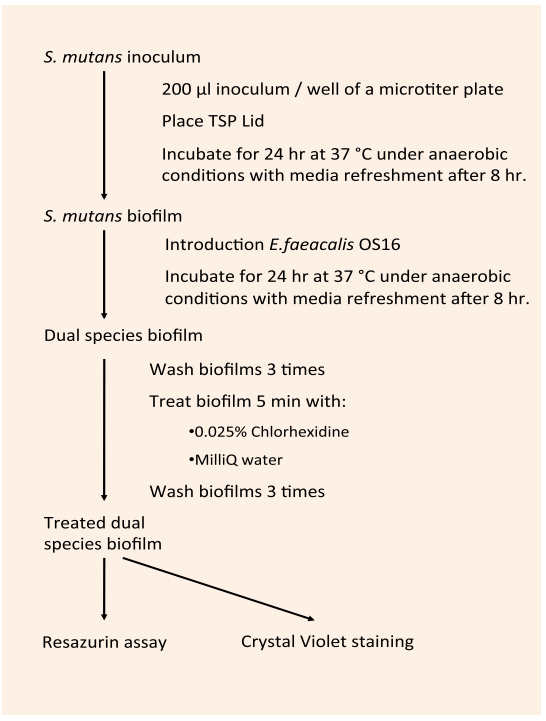
Aim

The aim of the study was to assess the influence of *Enterococcus faecalis* OS16 on the biofilm development and antimicrobial susceptibility of seven *Streptococcus mutans* strains towards a sub-lethal 0.025% chlorhexidine (CHX) 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.

See the flowchart for more details



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.

Figure 1
Reduction in biomass of dual species biofilm in the presence of E. faecalis *OS16 when compared to single species* S. mutans *biofilm. All data is significantly different (p<0.05).*

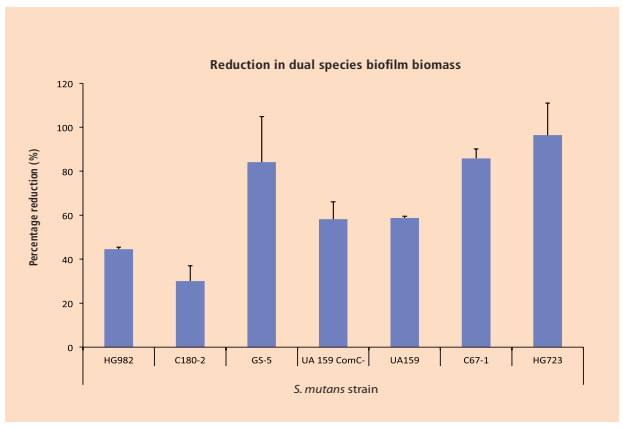
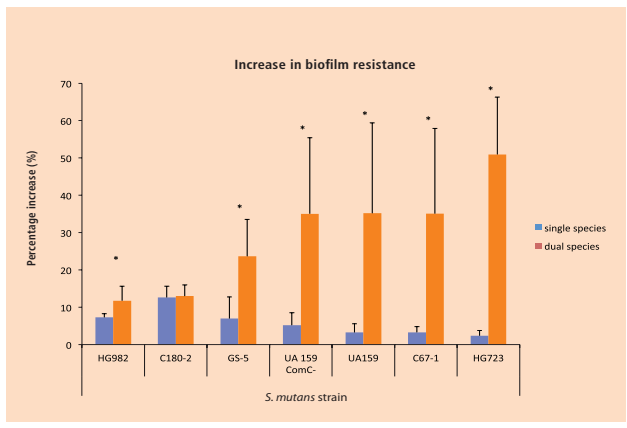


Figure 2
Percentage increase in resistance of dual species biofilm when compared to single S. mutans *biofilm after a 5 minute 0.025% CHX treatment. Data marked with a * is statistic significantly different (p<0.05).*



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.

Miller WD. The micro-organisms of the human mouth. Biel: Graphische Anstalt Schuler AG., 1890

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* Corresponding Author: Ing. M.A. Hoogenkamp
Academic Centre for Dentistry Amsterdam (ACTA)
University of Amsterdam and VU University Amsterdam
Department of Preventive Dentistry, Room 13N-37
Gustav Mahlerlaan 3004, 1081 LA Amsterdam
M.Hoogenkamp@acta.nl