The effect of glucose on *Streptococcus mutans* invasion of an *in vitro* synthetic community of oral bacteria

Jay S. Sangha[1],Thomas P. Curtis[1], Nicholas S. Jakubovics[2], Aline Metris[3], Paul Barrett[3], Irina D. Ofiteru[1]

[1]School of Engineering, Newcastle University, Newcastle upon Tyne,NE1 7RU; E-mail : j.sangha2@ncl.ac.uk
[2]School of Dental Sciences, Oral Biology, Newcastle University, Newcastle upon Tyne NE2 4BW,
[3]Safety and Environmental Assurance Centre, Unilever, Bedford MK44 1LQ.

Introduction

- One of the roles of a healthy oral microbiome is to impede colonisation by pathogenic species [1], preventing the development of a more aciduric and acidogenic populated biofilm.
- This work aimed to understand and quantify the functions underpinning colonisation of the oral microbiome by the cariogenic pathogen *Streptococcus mutans.*
- We hypothesise that altering environmental influences, such as lowering the concentration of glucose, will shift the ecology of the biofilm. In this instance, preventing the overgrowth of *S. mutans*, leading to a more balanced population of bacteria.

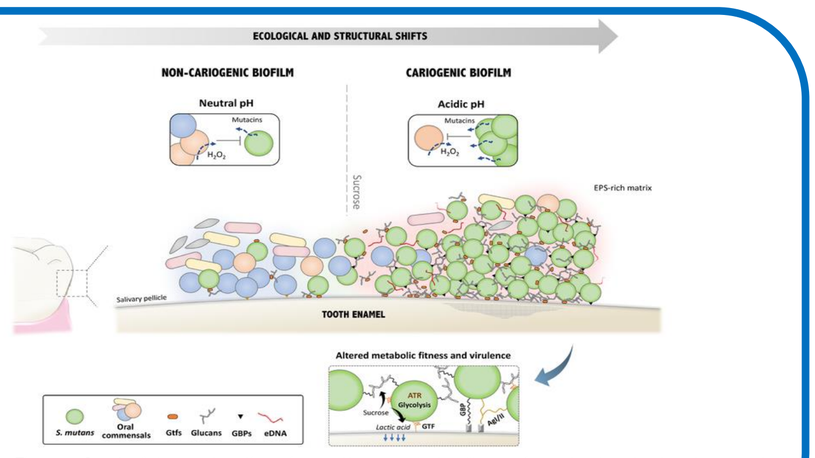

Figure 1. Acid production from the metabolism of carbohydrates by early colonisers shifts the pH and environment. Pathogens e.g. *S. mutans* then grow favourably, contributing to caries development [2].

Materials and methods

- The synthetic community of oral bacteria was grown in a chemically defined medium in CDC bioreactors. Reactors were run in triplicate for 9 days, and contained hydroxyapatite coupons for biofilm growth to mimic tooth enamel. The medium was fed in continuously at 0.4 mL min^{-1} , representative of salivary flow.
- *Actinomyces oris* MG1 was inoculated on day 0, with *Streptococcus gordonii* DL1, *Neisseria subflava* DSM17610 and *Veillonella parvula* DSM2008 on day 1. The pathogen, *Streptococcus mutans* UA159, was inoculated on day 2.
- Biofilm samples were collected on days 2 and 3, then every 48 hours until day 9. Biofilms were imaged using fluorescent *in situ* hybridization (FISH) and species were quantified using qPCR.

Results

- *S. mutans* colonised the biofilm within 24 hours of inoculation. At higher glucose concentration (20 g/L), *S. mutans* was the most prevalent species by day 9 (Figure 2A) .
- At lower glucose concentration (2 g/L), *S. mutans* was still able to invade the biofilm in high numbers, but the most dominant species was *V. parvula* (Figure 2B) , likely due to the abundance of lactic acid which it uses as its primary carbon source for growth.

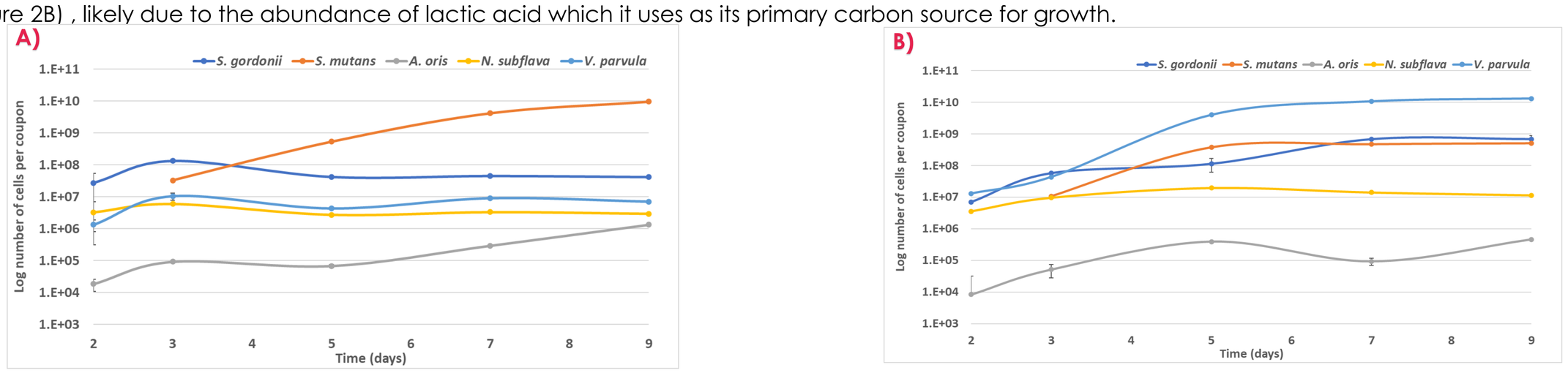

Figure 2. **A)** Growth of the synthetic community species over time at 20 g/L glucose concentration. *S. mutans* is the dominant species. **B)** Growth of the synthetic community species over time at 2 g/L glucose concentration. *V. parvula* is the dominant species.

- Growth of the synthetic community led to acid production, lowering the pH of the bulk medium. At the higher glucose concentration (20g/L), the pH reached an equilibrium of 5.3 , compared to 5.95 at the lower glucose concentration (Figure 3).

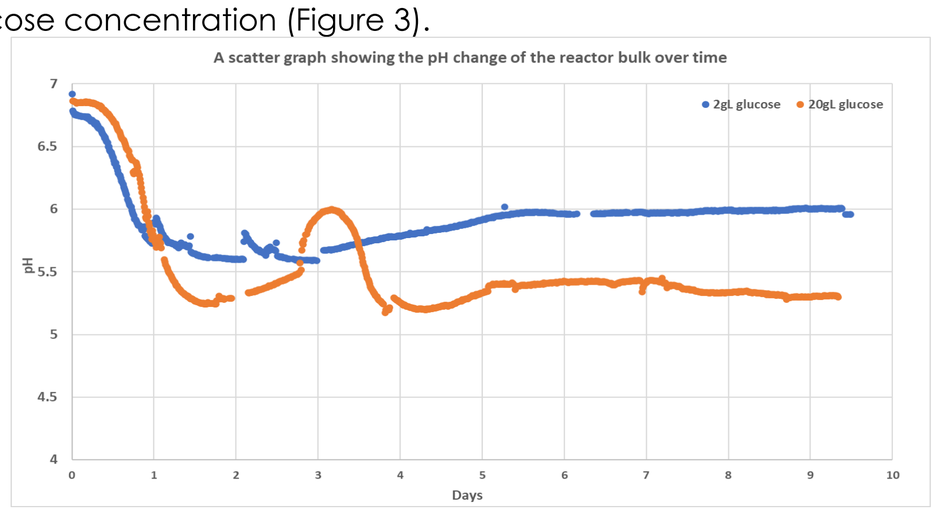

Figure 3. The pH shift in the planktonic bulk over time. As bacteria metabolise glucose, acid is produced and the pH drops.

- FISH images from day 9 show that *S. mutans* is prevalent in both a high sugar (Figure 4A) and low sugar (Figure 4B) environment, but there is a more balanced microbial ecology at lower sugar. *V. parvula* in particular is more prevalent, with the biofilm appearing denser in a low sugar environment.

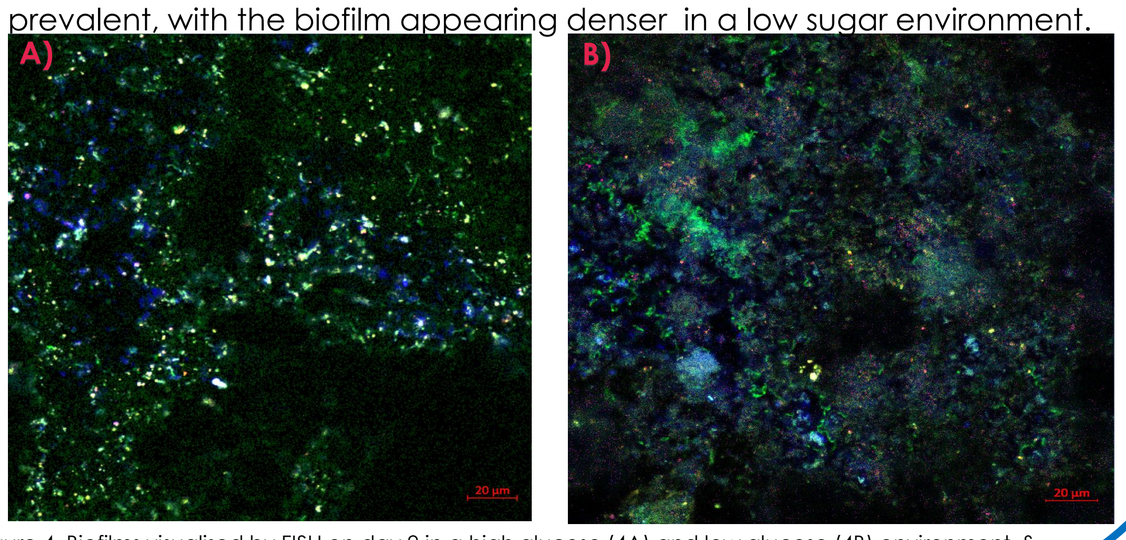

Figure 4. Biofilms visualised by FISH on day 9 in a high glucose (4A) and low glucose (4B) environment. *S. gordonii* (purple), *S. mutans (green), A. oris (yellow), N. subflava (blue), V. parvula (red).*

Conclusions

- We have successfully developed an *in vitro* model of the oral microbiome, where we have observed the invasion of *S. mutans* into a synthetic community.
- Our model demonstrates that a high glucose concentration enabled *S. mutans* to outcompete other oral bacteria in the biofilms, leading to a low pH environment, as seen *in vivo* [3].

References

1) McBain, AJ et al. *CMR*. 2019, 32 (4): 1-16
2) Lemos, JA et al. *Microbiol Spectr*. 2018, 7(1): 24-25
3) Featherstone, JDB. *ADA*, 2008, 53 (1): 286-290

Acknowledgements

This work is funded by the EPSRC- Unilever U.K. Ltd CASE project, number BH174538

EPSRC Pioneering research and skills

Unilever