

The development of a chemically defined medium for a synthetic oral microbial community and its characterisation with FISH

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Introduction

- ❖ The oral microbiome has over 700 species with overlapping metabolism and intricate physicochemical interactions, feeding on a complex medium.
- ❖ Colonisation by the pathogen *Streptococcus mutans* is often associated with caries development, costing globally over \$298 billion annually [1].
- ❖ We hypothesise that specific metabolic pathways maintain a “healthy” oral microbiome. Identifying critical pathways for health could contribute to better protection of the microbiome during oral care interventions.
- ❖ The aims were to define a representative synthetic community of oral bacteria as a proxy for the oral microbiome so it can be studied on a manageable scale, design a chemically defined medium (CDM) to untangle the influence of different substrates and develop fluorescence *in situ* hybridization (FISH) technique to visualise the spatial distribution of the community.

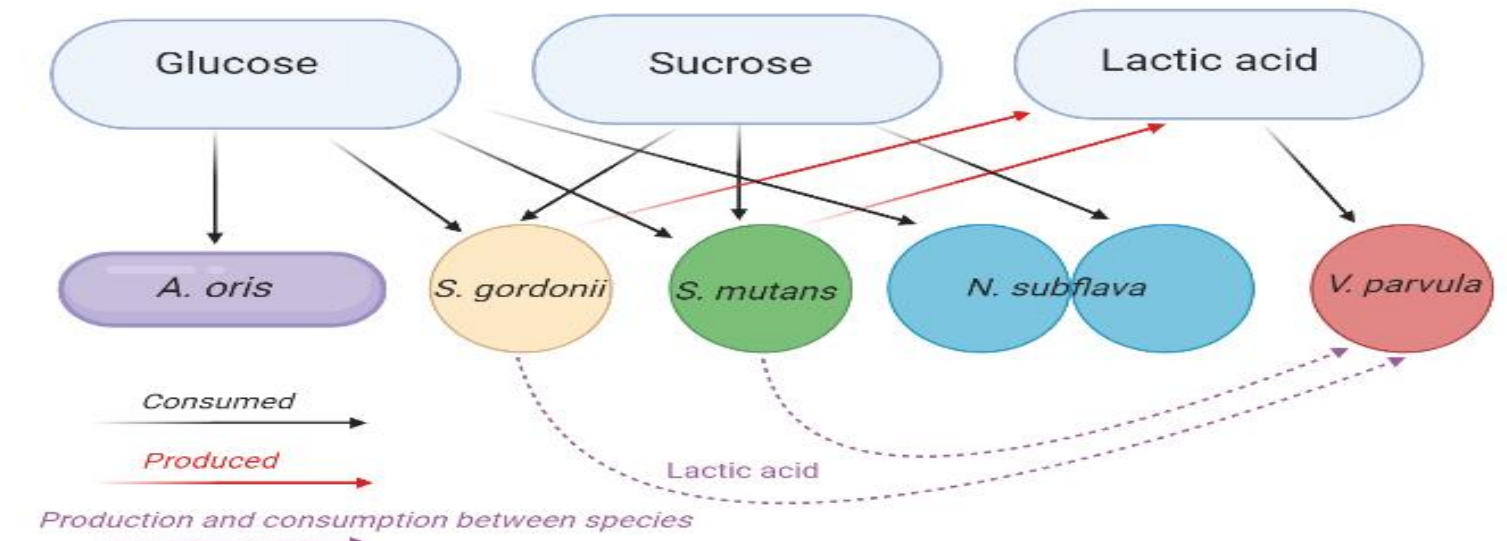


Figure 1. Metabolic interactions between chemically-defined medium and the synthetic community.

Materials and methods

- ❖ The bacterial strains used in this experiment, as part of an oral “synthetic community”, are: *Streptococcus gordonii* DL1, *Streptococcus mutans* UA159, *Actinomyces oris* MG1, *Neisseria subflava* DSM17610 and *Veillonella parvula* DSM2008. Strains were selected based on their metabolic function covering the main pathways of the oral microbiome, including sugar consumption and lactic acid production (Figure 1).
- ❖ All members of the synthetic community were grown on the same CDM, which was developed based on the FMC medium established by Terleckyi [2]. Modifications included the addition of lactic acid (1% v/v) in order to support the growth of *V. parvula*.
- ❖ Specific DNA FISH probes were identified and designed for each species of the synthetic community. Single species biofilms were cultured on glass slides, fixed, dehydrated, permeabilised and then hybridized for 3 hours at 55 °C. Samples were imaged using confocal laser scanning microscopy.

Results

- ❖ All members of the synthetic community grew successfully on the CDM when cultivated independently in suspension (Figure 2A). Glucose (20 g/L) and lactic acid (1% v/v) were the primary carbon sources present.
- ❖ Preferences for glucose and/or sucrose utilisation have been determined, exemplified by *S. gordonii* in Figure 2B. Glucose and sucrose are consumed simultaneously over 24 hours. At 5 hours, glucose concentration increases, possibly due to sucrose hydrolysing into glucose and fructose.

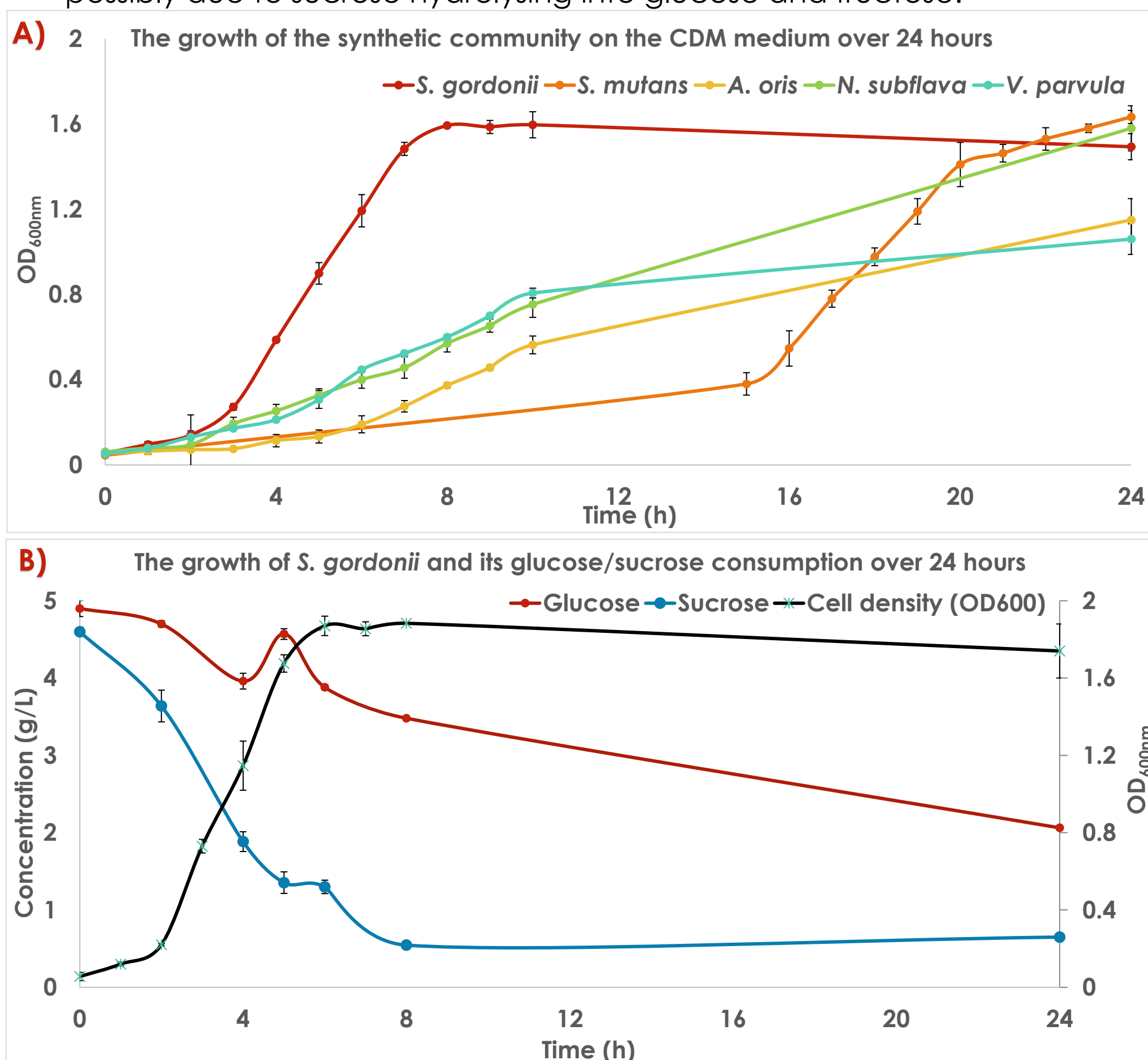


Figure 2. **A)** Growth of the synthetic community species on the CDM over 24 hours. **B)** Growth and glucose/sucrose utilisation of *S. gordonii* over 24 hours using the CDM. Both glucose and sucrose were present in the medium at the same time at an initial concentration of 5 g/L.

Biofilm visualisation

- ❖ All members of the synthetic community were successfully visualised using FISH as mono species biofilms, shown in Figure 3.

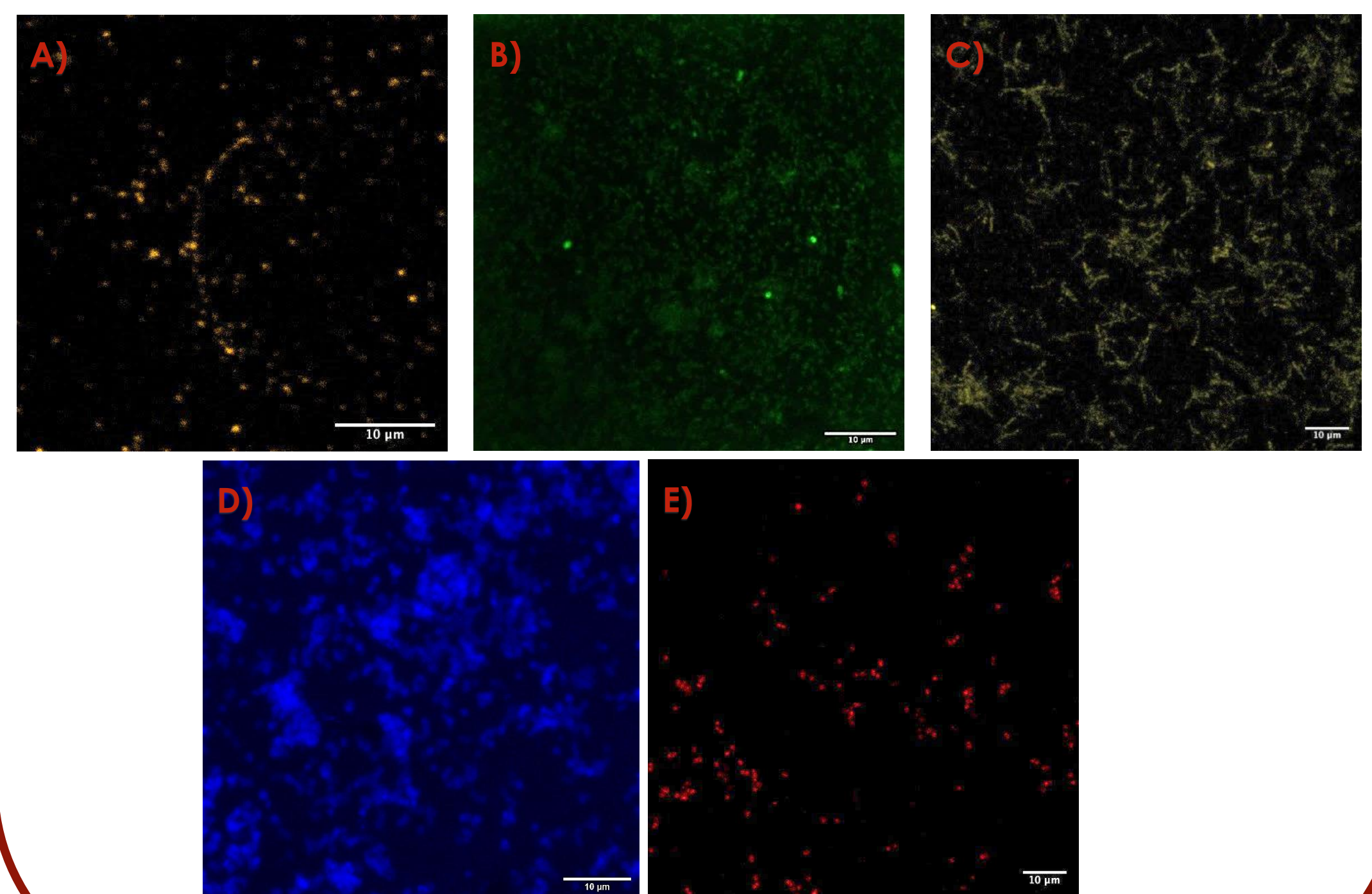


Figure 3. Members of the synthetic community visualised as monoculture biofilms using FISH. A) *S. gordonii*, B) *S. mutans*, C) *A. oris*, D) *N. subflava*, E) *V. parvula*.

Conclusions

- ❖ We have successfully developed a CDM that sustains growth of all members of the selected synthetic community.
- ❖ Members of the synthetic community utilise more than one carbon source at the same time.
- ❖ All species have been successfully visualised individually and this will be expanded to the whole synthetic community.
- ❖ These approaches will be used in the development of mathematical models of the system.

References

- 1) Listl, S. et al. *J Dent Res*, 2015, 94(10): 1355-1361.
- 2) Terleckyj, B. et al. *Infect Immun*. 1975, 11(4):649-655.

Acknowledgements

This work is funded by the EPSRC- Unilever U.K. Ltd CASE project, number RES/0530/7469.