Biofilm Formation by *Rothia mucilaginosa* and *Candida albicans* in Dual-Species is Significantly Enhanced

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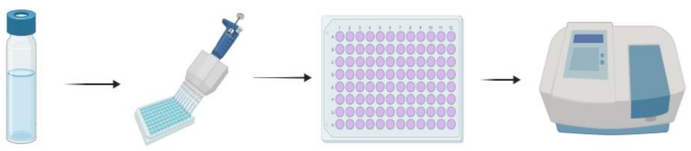
Introduction

- Interactions between the different major species in the oral microbiota are believed to play an important role in oral health and disease.
- A keystone pathogen of human dental caries, *Streptococcus mutans* can facilitate the colonization of *Candida albicans,* known to cause oral thrush, leading to rampant early childhood caries.
- *Rothia mucilaginosa* is one of the most abundant bacteria of the oral microbiota, but its role in oral health remains unclear.
- This study is designed to investigate if *R. mucilaginosa* interacts with *S. mutans* and *C. albicans,* influencing biofilm formation.

Methods and Materials

- **96 well-plate Biofilms:** Bacterial strains were grown individually in trypticase soy broth plus yeast extract at 0.1% (w/v) (TYE) and sucrose (2 mM), diluted and mixed properly, and then aliquots were loaded to 96 well culture plates. After incubation at 37°C for 24 hours, biofilms were stained with 0.1% crystal violet and measured using a spectrophotometer.



- **For glass slides model**, glass slides were deposited vertically in 50 mL culture tubes, and biofilms on the slides were briefly sonicated, and then serial dilutions were plated on agar medium for counting of colony-forming units (CFU).
- **Acid killing assay:** The bacterial strains were grown in 50 mL TSY broth, washed once with 0.1 M glycine buffer, pH 7.0, then incubated in 0.1 M glycine buffer, pH of 2.8 for a period of 0, 30, 45, and 60 minutes. Surviving cells were plated on agar medium, and acid tolerance in survival rate of the different species were calculated.

R. mucilaginosa and *C. albicans* enhanced their growth when grown together

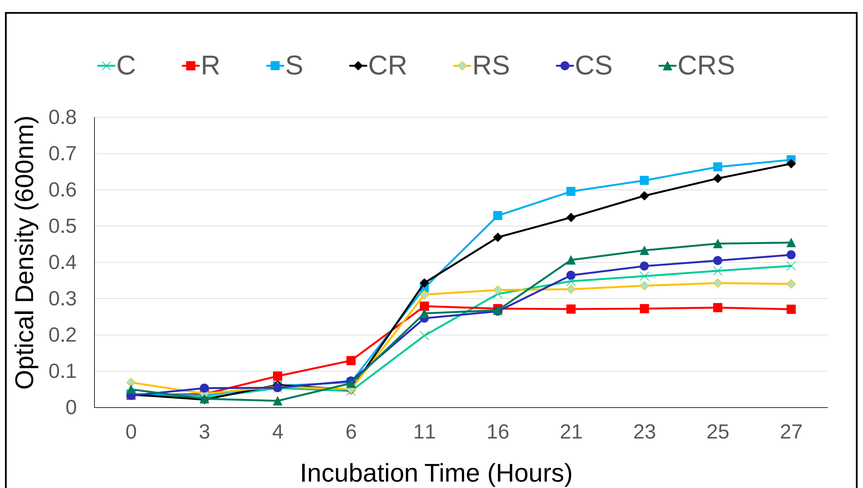


Fig. 1. Growth curve. *Candida* (C), *Rothia* (R), *Streptococcus* (S), *Rothia* and *Streptococcus* (RS), *Candida* and *Streptococcus* (CS), *Candida*, *Rothia,* and *Streptococcus* (CRS) were grown in TYE.

Biofilms by *Rothia* and *Candida* were enhanced when grown in dual-species

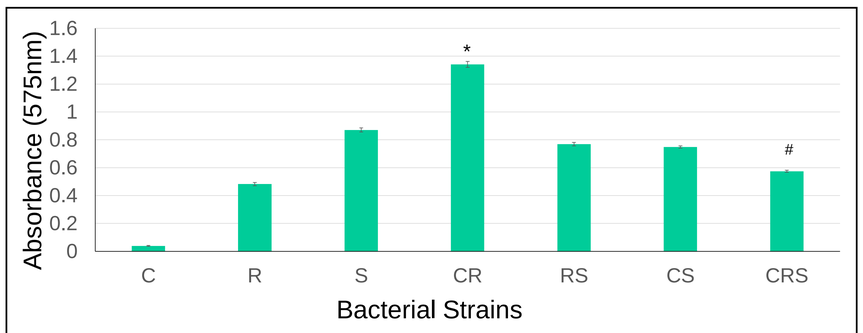


Fig. 2. 24-hour biofilms in 96 well-plates. Student *t*-test *, $P<0.001$ vs C and R; #, $P<0.05$ vs S. See Fig. 1 for abbreviations.

S. mutans and *C. albicans* increased biofilms when grown together

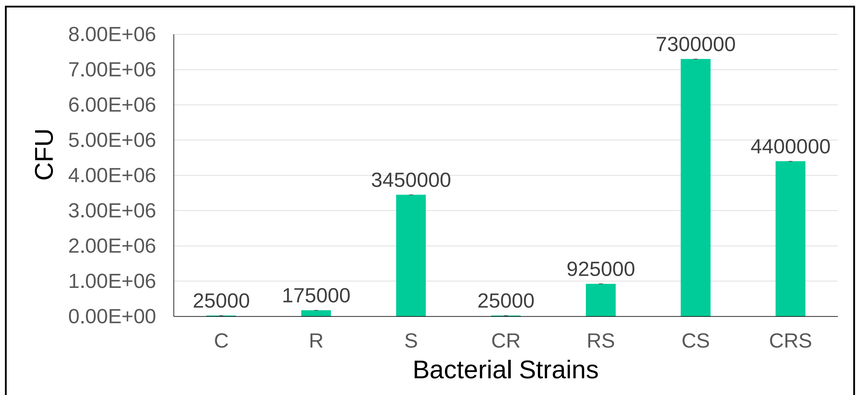


Fig. 3. Biofilm formation on glass slides. Data shows results in colony-forming-units of 24-hour biofilms. See Fig. for Abbreviations.

pH profile of cultures grown alone and in mixed-species model

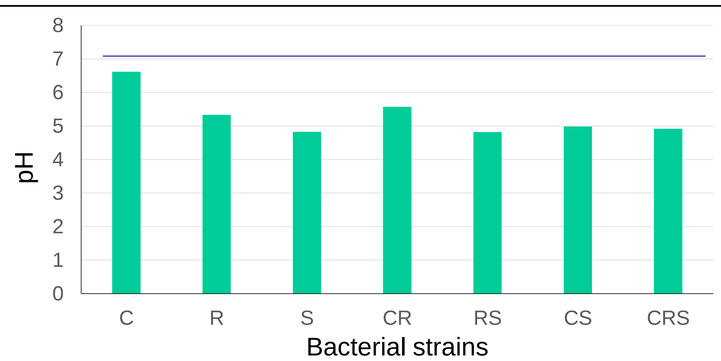


Fig. 4. pH profile. The pH values of the 27-hour cultures were analyzed using a pH probe. The blue line indicates the starting pH at 7.15.

Differences in acid tolerance exist between the microbes

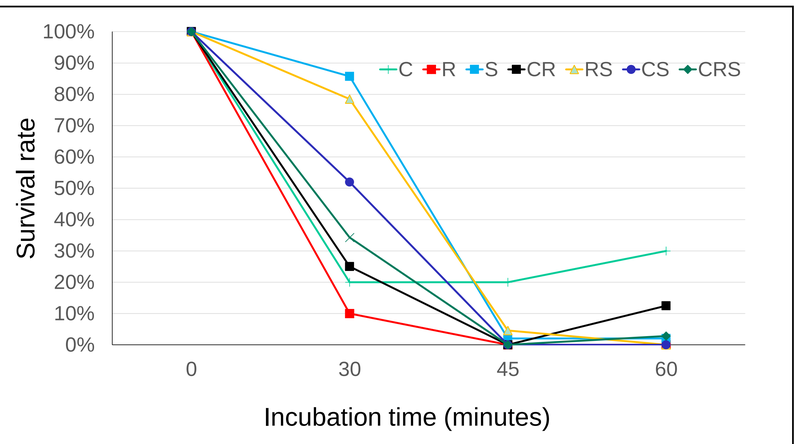


Fig. 5. Acid killing assay. Survival rate is expressed as percentage of surviving cells at each time point over the initial cell numbers at time 0. See Fig. 1 for abbreviations.

Discussion

- *S. mutans* formed the most biofilms in 96 well plates, while *C. albicans* had the least, when grown individually.
- *C. albicans* and *R. mucilaginosa* in dual-species increased biofilm formation, compared to the respective mono-species model. This can be in part attributed to the enhanced growth, when grown together.
- These results suggest that *C. albicans* and *R. mucilaginosa* interact influencing biofilm formation, although the exact underlying mechanism awaits further investigation.

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