Effects of Polymicrobial Interactions on Porphyromonas *gingivalis* Biofilm Formation

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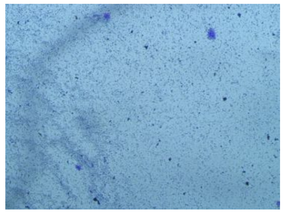
Introduction

- Periodontitis is a polymicrobial inflammatory disease caused by disruptions in the oral microbial community.
- Within these communities, bacteria interact through secreted extracellular products, including proteins, metabolites, and outer membrane vesicles (OMVs) that can influence microbial behavior.
- Porphyromonas *gingivalis* (Pg) is a keystone periodontal pathogen that contributes to disease progression by altering host immune responses and microbial community structure.
- The mechanisms by which interactions between Pg and neighboring bacterial species influence biofilm formation and extracellular product production remain poorly understood.
    - A biofilm is a community of microorganisms attached to a surface and embedded within a self-produced extracellular matrix.

A.



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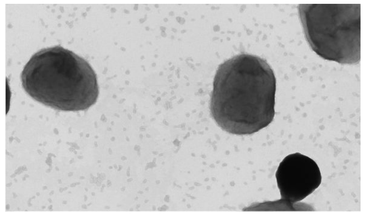


Figure 1. Representative images of biofilm formation and polymicrobial growth. (A) Crystal violet-stained P. *gingivalis* monoculture biofilm. Direct magnification: 10,000x; scale: 500 nm. **(B)** Transmission electron microscopy (TEM) image of P. *gingivalis* and B. *thetaiotaomicron* co-culture showing bacterial cells and extracellular vesicles. Direct magnification: 10,000x; scale: 500 nm.

Significance

Understanding bacterial communication within polymicrobial communities may reveal mechanisms contributing to periodontal disease progression and identify potential targets for disrupting pathogenic biofilms.

Hypothesis

Polymicrobial interactions alter Pg biofilm formation through changes in bacterial community behavior and extracellular product production.

Methods and Results

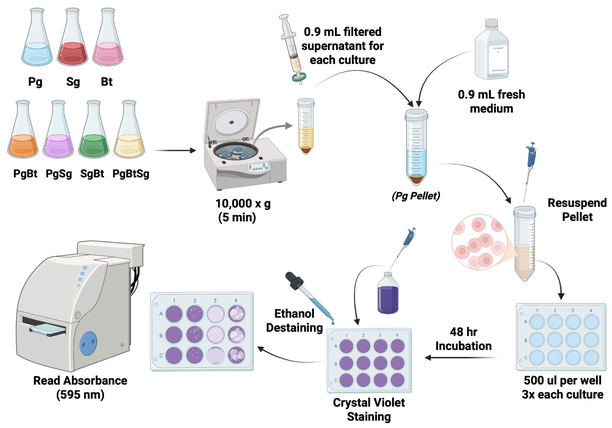
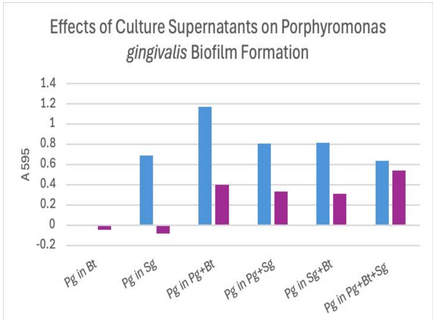


Figure 2. Biofilm Formation Assay. Mono-, di-, and polymicrobial cultures were grown under biofilm-forming conditions. Biofilm biomass was quantified using crystal violet staining.

A.



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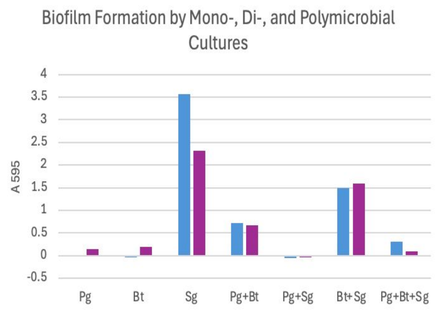


Figure 3. Polymicrobial interactions increase *P. gingivalis* biofilm formation.
(A) The effects of bacterial culture supernatants on *P. gingivalis* biofilm formation were evaluated by exposing *P. gingivalis* biofilms to supernatants collected from mono- and polymicrobial cultures. Biofilm formation was normalized to the Pg monoculture, therefore, the Pg control is not shown. Differences in normalized biofilm biomass indicate that secreted bacterial products influence P. *gingivalis* biofilm development. Blue and pink bars represent two independent biological replicates (n = 2). **(B)** Biofilm formation was assessed in mono-, di-, and polymicrobial cultures to evaluate how bacterial community composition affects biofilm development. Biofilm formation increased in the Pg+Bt and Bt+Sg co-cultures compared with Pg and Bt monocultures, while Pg+Sg and Pg+Bt+Sg showed decreased biofilm formation compared with Sg monoculture. Blue and pink bars represent two independent biological replicates (n = 2).

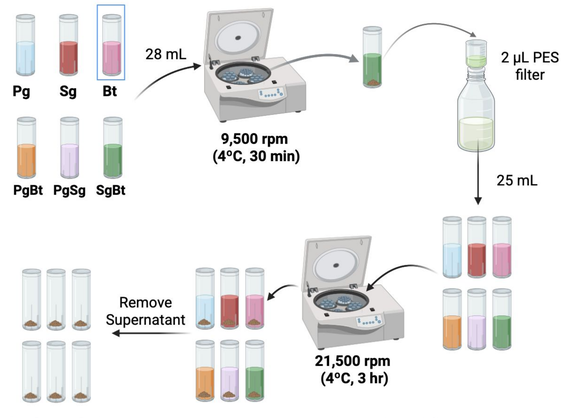


Figure 4. OMV Isolation. Culture supernatants were collected and from mono- and polymicrobial cultures, and OMVs were isolated for downstream analysis.

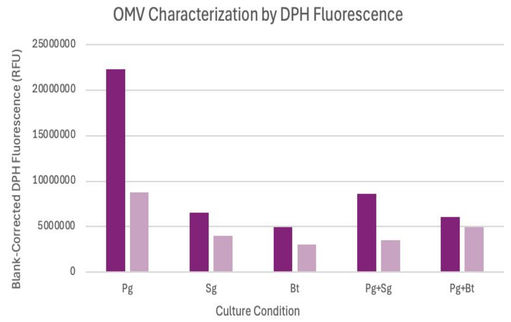
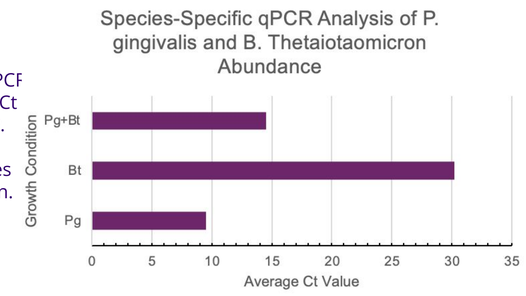


Figure 5. DPH fluorescence analysis of extracellular vesicle-associated membrane content from mono- and polymicrobial cultures. Blank-corrected DPH fluorescence was compared across OMVs isolated from mono- and polymicrobial cultures. Pg showed the highest fluorescence, while polymicrobial conditions showed lower or variable fluorescence, indicated differences in vesicle-associated membrane content. Sets 1 and 2 represent two independent OMV isolations (n = 2).

Figure 6. Species-specific qPCR analysis of *P. gingivalis* and B. *thetaiotaomicron* abundance during mono- and di-microbial growth. Species abundance was assessed using species-specific qPCR targeting the *mfa1* gene. Average Ct values were compared between P. *gingivalis* monoculture and B. *thetaiotaomicron* (Bt) monocultures and the Pg+Bt co-culture condition. Higher Ct values indicate lower detection of the corresponding bacterial target. Pg exhibited a higher Bt value in co-culture compared with Pg monoculture, indicating reduced Pg abundance during polymicrobial growth. Bars represent the mean Ct value from eight replicate measurements (n = 8) for each growth condition.



Key Findings & Future Directions

- Polymicrobial interactions increased *P. gingivalis* biofilm formation and bacterial community behavior.
- Culture supernatants from mono- and polymicrobial conditions influenced *P. gingivalis* biofilm development, suggesting a role for secreted extracellular products.
- OMVs were isolated and characterized using DPH fluorescence, supporting their potential role in bacterial communication.
- Reduced *P. gingivalis* abundance during co-culture with *B. thetaiotaomicron* suggests that increased biofilm formation is not solely due to increased bacterial growth.
- Further OMV characterization is needed to identify vesicle-associated components responsible for altered *P. gingivalis* behavior.
- Future studies will evaluate how extracellular products influence bacterial interactions and host-pathogen responses during periodontal disease progression.

References

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