A Tale of Two Strains

Comparative analysis of two closely related *Porphyromonas gingivalis* wild-type strains and their outer membrane vesicles

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Background

Porphyromonas gingivalis (Pg) is a keystone pathogen in chronic periodontitis (gum disease). 33277 and 381 are two commonly studied wild-type strains of *P. gingivalis*. Both represent naturally occurring strains isolated from human periodontal samples. Genomically, they are nearly identical but exhibit different pathogenicity profiles.

Contributors to Virulence

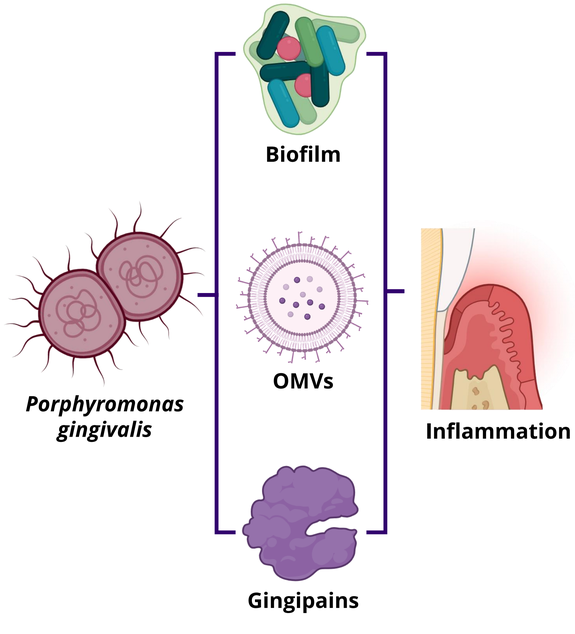
Biofilm Formation. Aggregation of bacteria to a surface and facilitate host colonization by adhering to tooth surfaces.

Outer Membrane Vesicles (OMVs). Nanoparticle delivery system that contributes to interbacterial and host interactions by carrying cargo including gingipains, genetic material, and other virulence factors formed from the outer membranes of gram-negative bacteria.

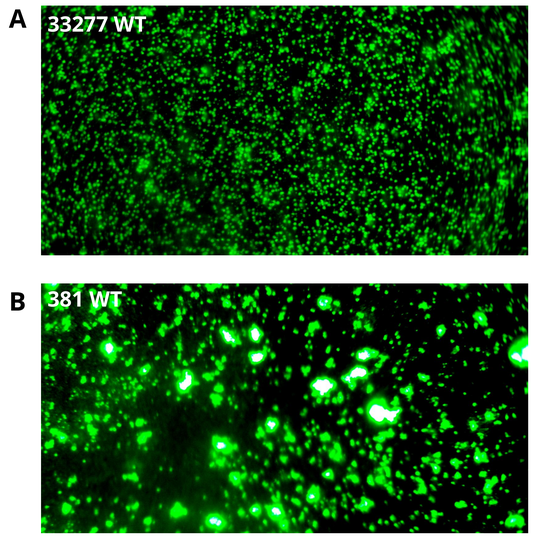
Gingipains. Proteolytic enzymes contribute to inflammation in the host by stimulating pro-inflammatory cytokines to further pathogenesis.

Hypothesis

The observed differences in immunostimulatory capabilities of 33277 and 381 from prior studies are linked to variations in pathogenicity profiles.

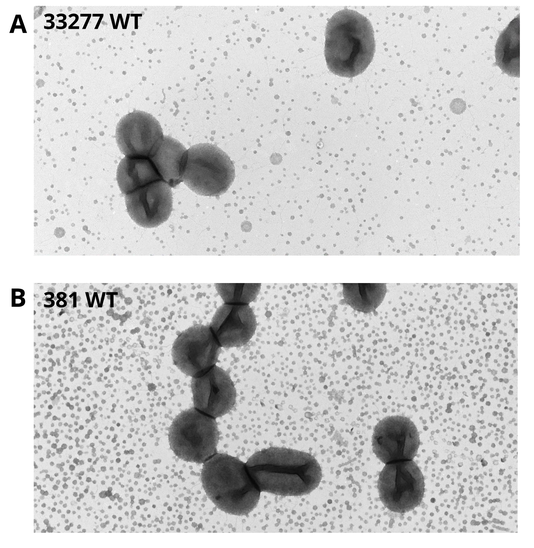


Biofilm morphologies differ, despite similar cell densities



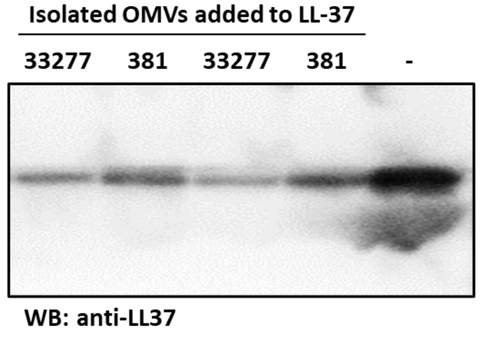
Epifluorescence images of CFSE-stained biofilms formed by common *Pg* lab strains 33277 **(A)** and 381 **(B)** reveal differences in cell arrangement, whereas the strains accumulate similar cell biomasses **(C)**, measured with pixel intensity of images and averaged (n=3).

Pg 381 WT produces more OMVs than *Pg* 33277 WT



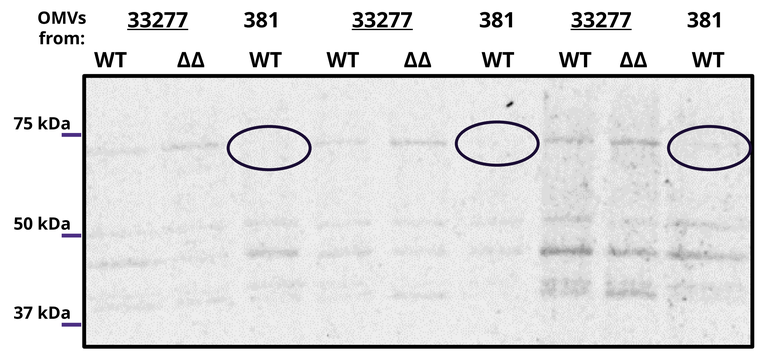
TEM images of 33277 **(A)** and 381 **(B)** whole broth cultures, in addition to total protein quantification of isolated outer membrane vesicles with Bicinchoninic Acid (BCA) assay (n=3) **(C)** reveal differences in the abundance of produced OMVs by each strain, where *Pg* strain 381 produces more OMVs than 33277.

Gingipain activity remains reduced in *Pg* 381 WT OMVs, independent of OMV abundance



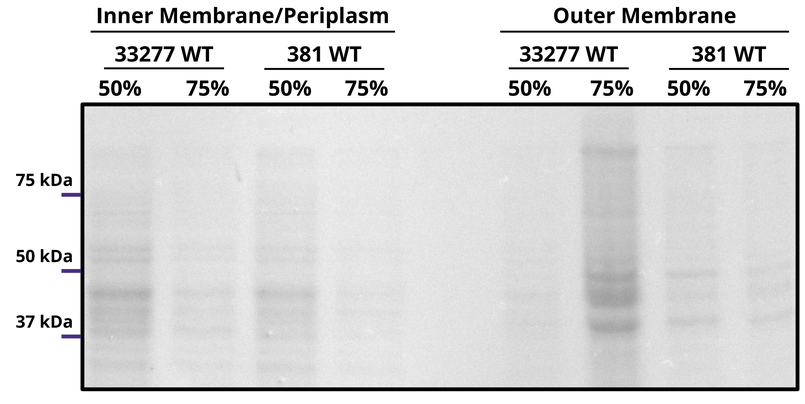
OMVs were isolated from two independent sets of cultures that both included Pg 33277 WT and *Pg* 381 WT. 45 µl of isolated OMVs resuspended in 1X sterile PBS were added to 5 µg of human LL37 peptide and incubated for 30 minutes at 37°C. Protease activity was determined via degradation of LL37, Western Blot detected undegraded LL37.

SDS-PAGE reveals a difference in protein OMV cargo between the two strains



OMVs were isolated from three independent sets of cultures, each including *Pg* 33277 WT, *Pg* 33277 ΔΔ, and *Pg* 381 WT. Protein concentrations of OMVs were determined by BCA assay and normalized to the lowest in each set. SDS-PAGE gel stained with Coomassie Blue of OMVs revealed the presence of a band at ~73kDa in *Pg* 33277 OMVs, and absent in *Pg* 381 WT.

Outer Membrane isolation can be used to study cargo loading of OMVs by each strain



Outer membrane fractions of *Pg* 33277 WT and *Pg* 381 WT broth cultures were grown to approximate log phase, mechanically lysed with either 50% or 75% sonication, and subjected to Sarkosyl-insoluble outer membrane preparation and SDS-PAGE stained with Coomassie Blue.

Ongoing work

- Gain a better understanding of the functional differences between OMVs produced by these two strains
- Use comparative proteomics to characterize differences between 33277 and 381 outer membrane and OMV proteomes
- Understand these proteins role in the observed differences in virulence

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Coats, *et al*., 2019. doi: 10.1128/IAI.00319-19
Gutner, *et al.*, 2009. doi:10.1128/IAI.00648-09

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