

Porphyromonas gingivalis Outer Membrane Vesicles Modulate Biofilm Morphology



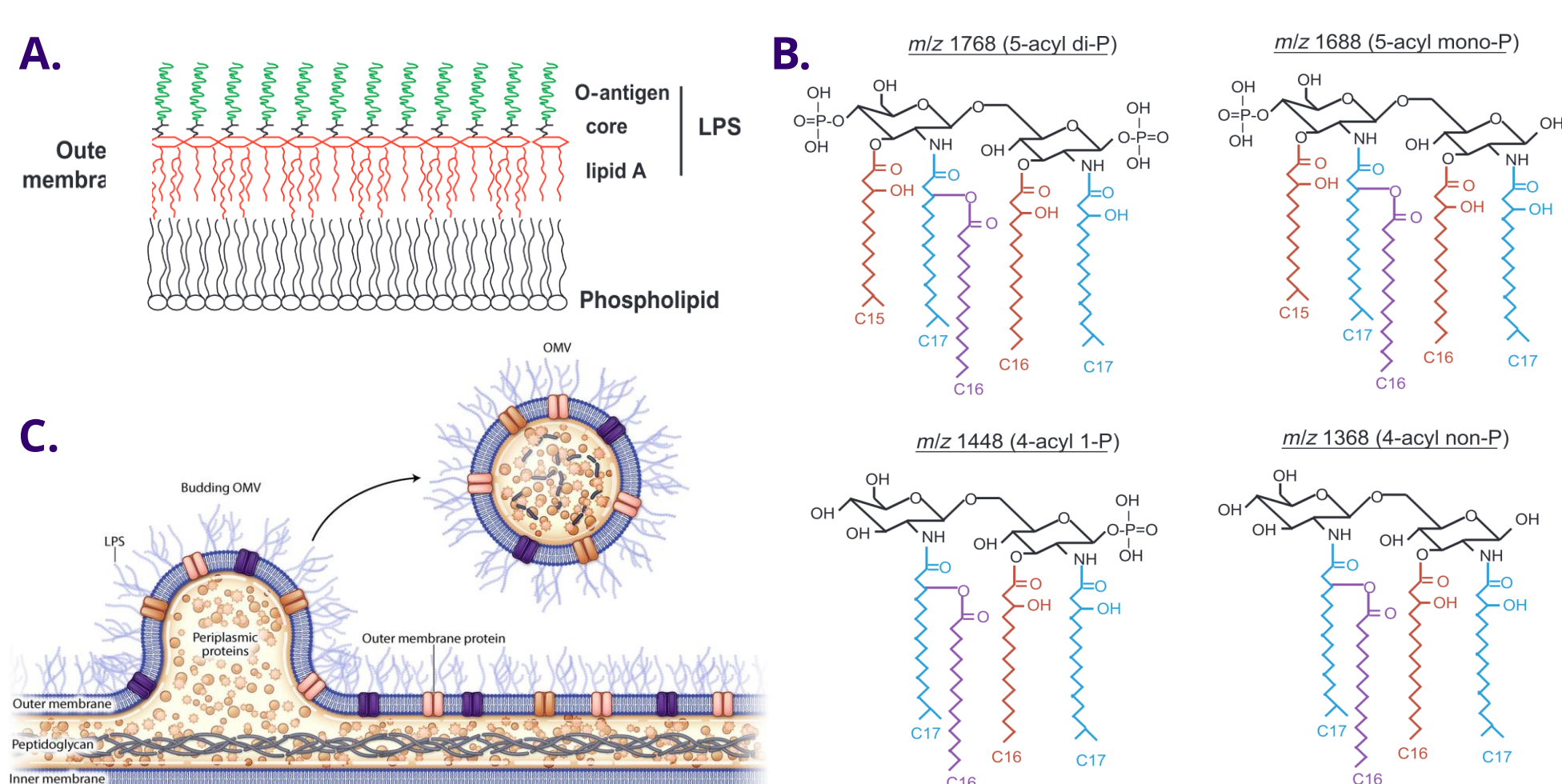
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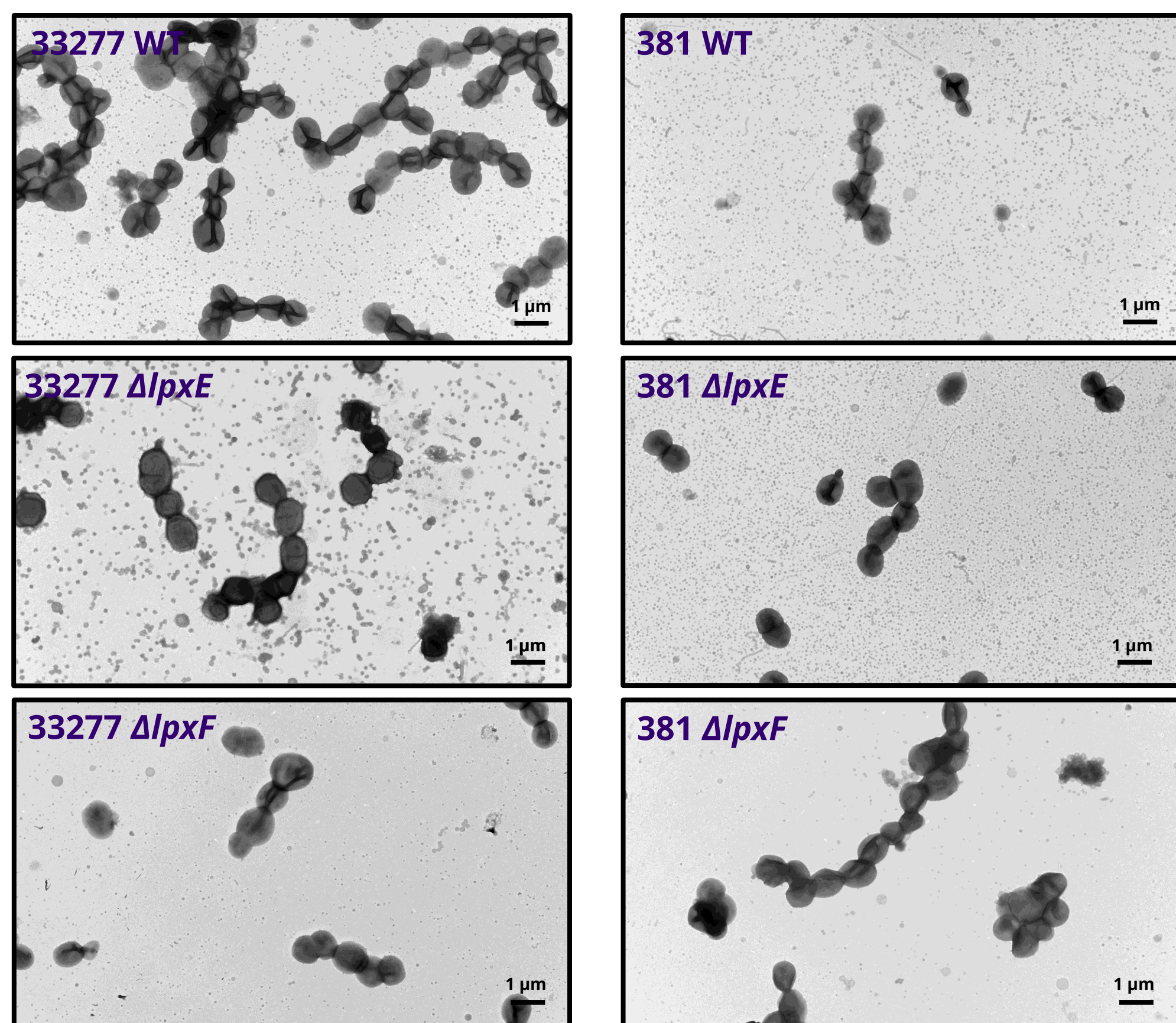
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Introduction

- *Porphyromonas gingivalis* (*Pg*) is a Gram-negative keystone pathogen in chronic periodontitis.
- Produces abundant outer membrane vesicles (OMVs), nanosized vesicles that transport a diverse range of cargo that play a role in biofilm regulation.
- We compared the almost genetically identical *Pg* wild type (WT) strains 33277, 381, and their isogenic mutants: $\Delta lpxE$, $\Delta lpxF$, and $\Delta lpxZ$.



The outer membrane component lipid A is modified in *Pg*. (A) Schematic of outer membrane leaflet. (B) Lipid A structures found in *Pg*. WT outer membrane (OM) consists of a mixture of 1448 and 1368, $\Delta lpxE$ with 1448, $\Delta lpxF$ with 1688, and $\Delta lpxE/F$ with 4-acetyl di-P lipid A. (C) Schematic of OM vesiculation.

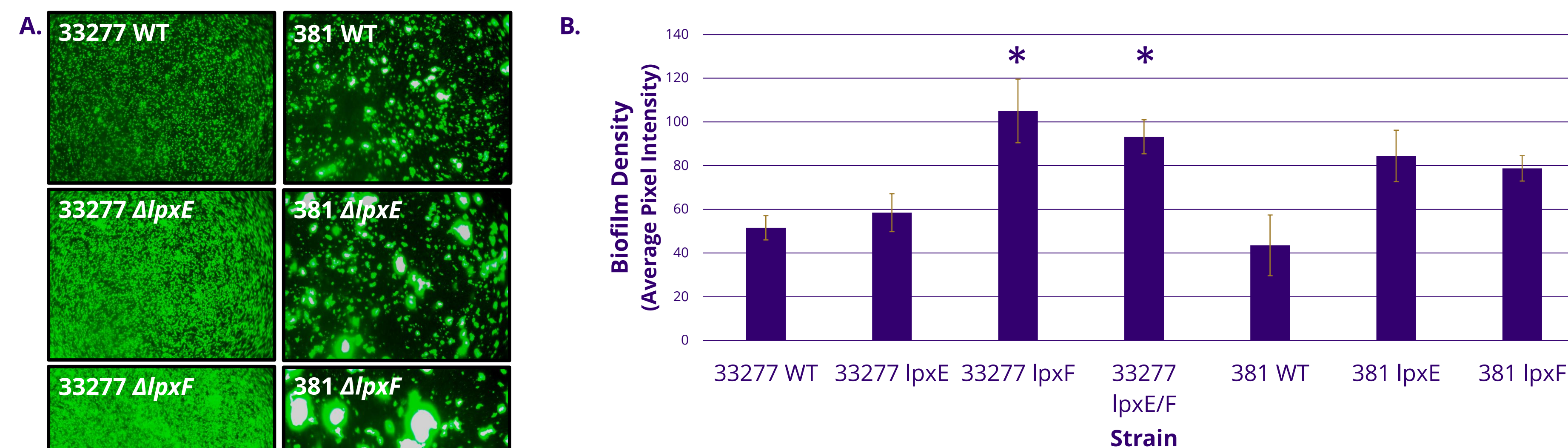


WT *Pg* 381 produces more abundant OMVs than WT *Pg* 33277. TEM images of whole broth cultures, in addition to quantification of isolated OMVs (not shown) reveal differences in the abundances of OMVs produced by two closely related WT *Pg* strains.

Hypothesis

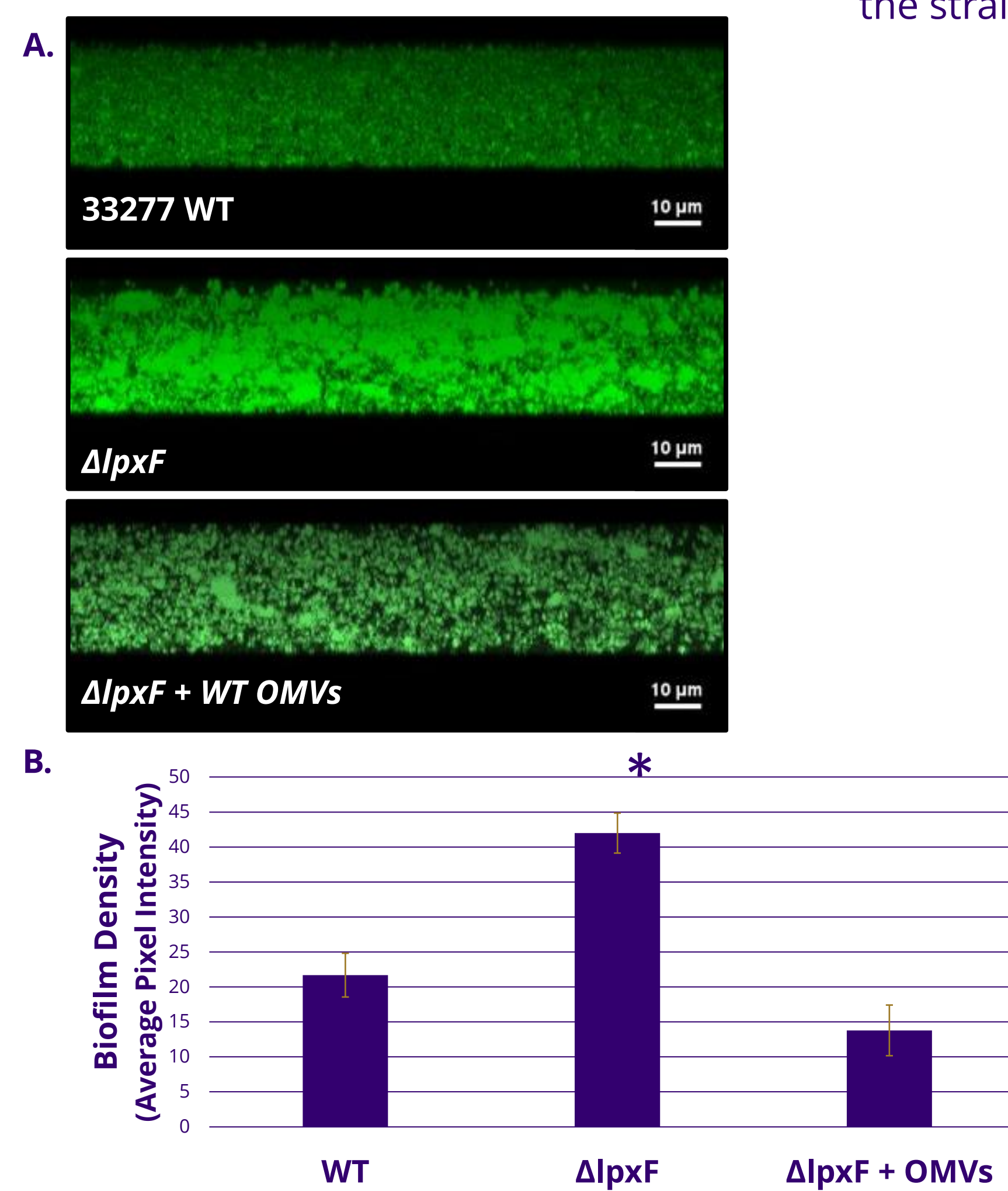
Differences in the abundance and/or contents of OMVs produced by *Pg* 381 and *Pg* 33277 result in distinct biofilm structures formed by each strain.

Comparison of Biofilm Morphology



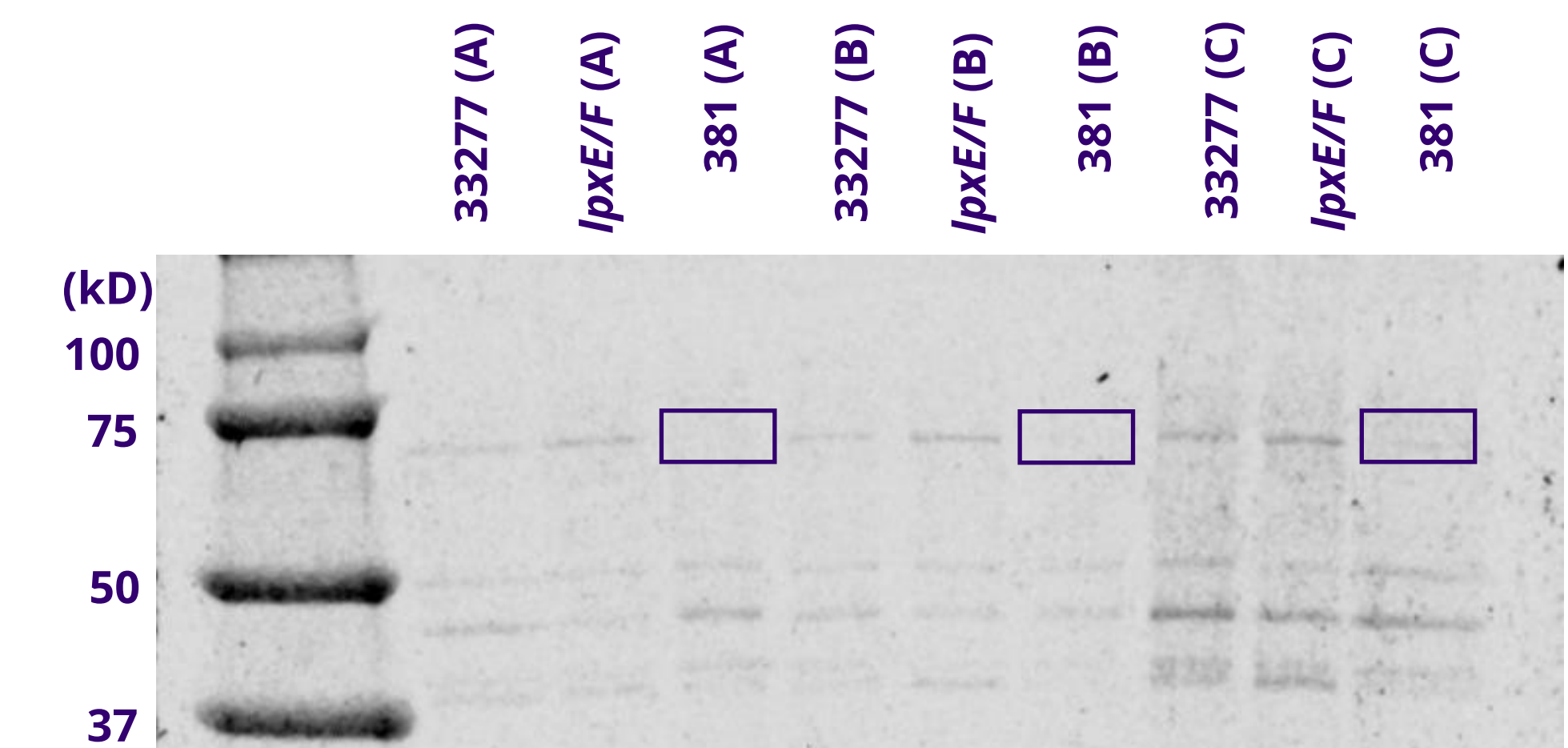
Biofilm morphologies of WT 33277 and WT 381 strains differ, despite similar total cell densities. 33277 *lpxF* differs significantly from WT, higher cell density.

(A) Epifluorescence images of CFSE stained biofilms formed by WT *Pg* strains 33277 and 381 reveal differences in cell arrangement, whereas the strains accumulate similar cell biomasses overall (B).

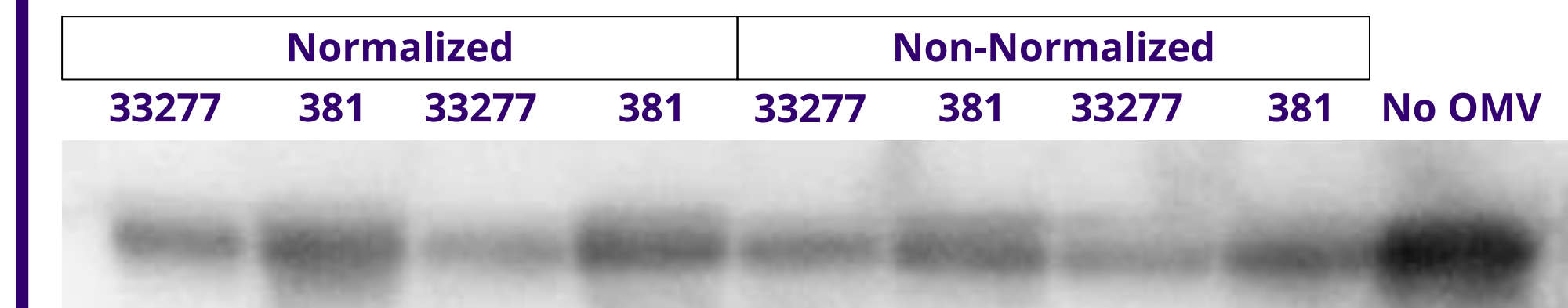


OMVs modulate biofilm density in *Pg*. (A) Confocal images of CFSE stained biofilms formed by 33277 WT and $\Delta lpxF$ reveal differences in biofilm density. Addition of WT OMVs to $\Delta lpxF$ cells causes a lower density biofilm to form compared to $\Delta lpxF$ without added OMVs. (B) Data are representative of two biological replicates.

Strain Specific OMV Differences



Protein composition of OMVs differs between *Pg* 381 and *Pg* 33277. OMVs were isolated from three independent sets of cultures that included WT 33277, 33277 $\Delta lpxE/F$, and WT 381 in each set. Protein concentrations of isolated OMVs were determined by BCA assay and normalized to the lowest concentration in each set, then OMVs were subjected to SDS-PAGE and the gel was stained with Coomassie blue.



Protease activity is reduced in *Pg* 381 OMVs compared to *Pg* 33277 OMVs. OMVs were isolated from two independent sets of cultures that included *Pg* 33277 and *Pg* 381 in each set. *Pg* 381 OMV protein concentrations were either normalized to *Pg* 33277 or not, and then 45 μ l of OMVs were added to 5 μ g of human LL37 peptide to detect protease activity. After 30 minutes at 37°C, undegraded LL37 remaining in each sample was detected by Western blotting.

Future Studies

- Identify the OMV protein(s) that differ in abundance between *Pg* 33277 and *Pg* 381 and determine if these proteins contribute to biofilm formation, dispersal etc.
- Use comparative proteomics to identify differences in outer membrane and/or OMV proteomes in *Pg* 381 and *Pg* 33277 that may contribute to differences in OMV abundance and biological function.
- In-depth characterization of the roles of *Pg* OMVs in regulation of biofilm structure and function, especially in the context of multi-species bacterial communities.

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References

- Coats SR, Kantrong N, To TT, Jain S, Genco CA, McLean JS, Darveau RP. 2019. The Distinct Immune-Stimulatory Capacities of *Porphyromonas gingivalis* Strains 381 and ATCC 33277 Are Determined by the fimB Allele and Gingipain Activity. *Infection and Immunity*. 87(12):e00319-19. doi:10.1128/IAI.00319-19.
- Jain S, Darveau RP. 2010. Contribution of *Porphyromonas gingivalis* lipopolysaccharide to periodontitis. *Periodontology* 2000. 54(1):53-70. doi:10.1111/j.1600-0757.2009.00333.x.
- Lieberman LA. 2022. Outer membrane vesicles: A bacterial-derived vaccination system. *Frontiers in Microbiology*. 13. [accessed 2023 Nov 27]. <https://www.frontiersin.org/articles/10.3389/fmicb.2022.1029146>.

