



UNIVERSITY OF
BIRMINGHAM

COLLEGE OF
MEDICAL AND
DENTAL SCIENCES

 @MuchovaMaria

Fusobacterium nucleatum subspecies differ in biofilm forming ability *in vitro*

Maria Muchova

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Muchova M, Balacco DL, Grant MM, Chapple ILC, Kuehne SA and Hirschfeld J (2022) *Fusobacterium nucleatum* Subspecies Differ in Biofilm Forming Ability *in vitro*. *Front. Oral. Health* 3:853618. doi: 10.3389/froh.2022.853618

Fusobacterium nucleatum

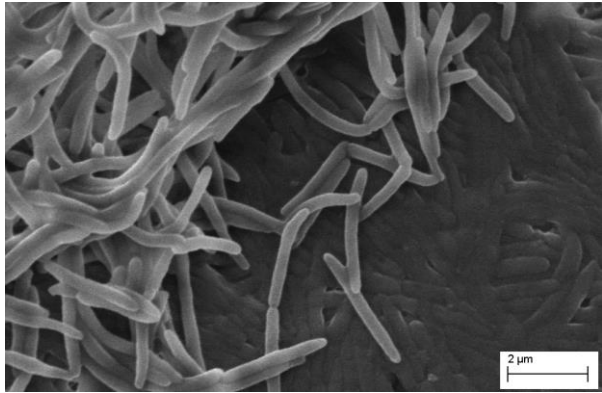
Commensal Gram-negative oral anaerobe¹

Key bridging species in oral biofilms²

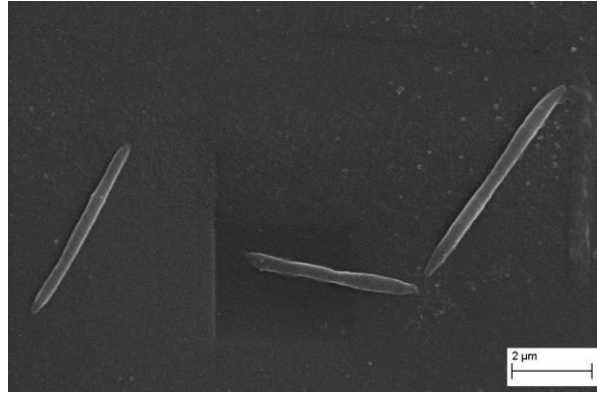
Opportunistic pathogen³

Adhesins mediating coaggregation and biofilm formation: RadD and Aid1, Fap2, FomA, CmpA⁴, FadA⁵, YadA-like adhesin⁶

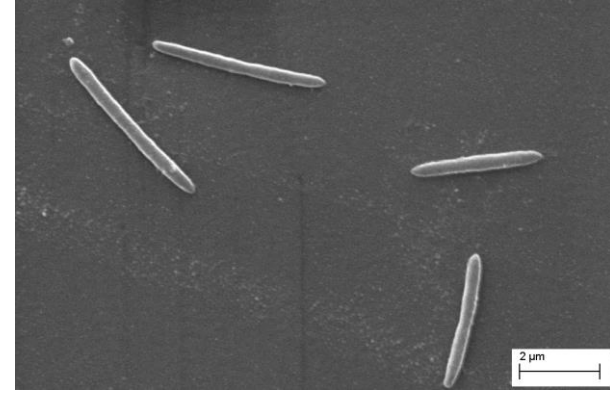
Fusobacterium nucleatum subspecies



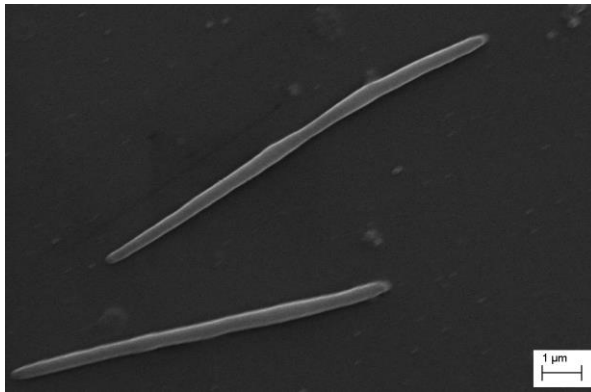
animalis (FNA)



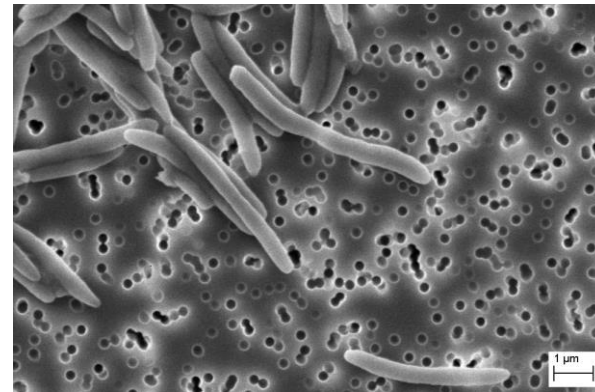
fusiforme (FNF)



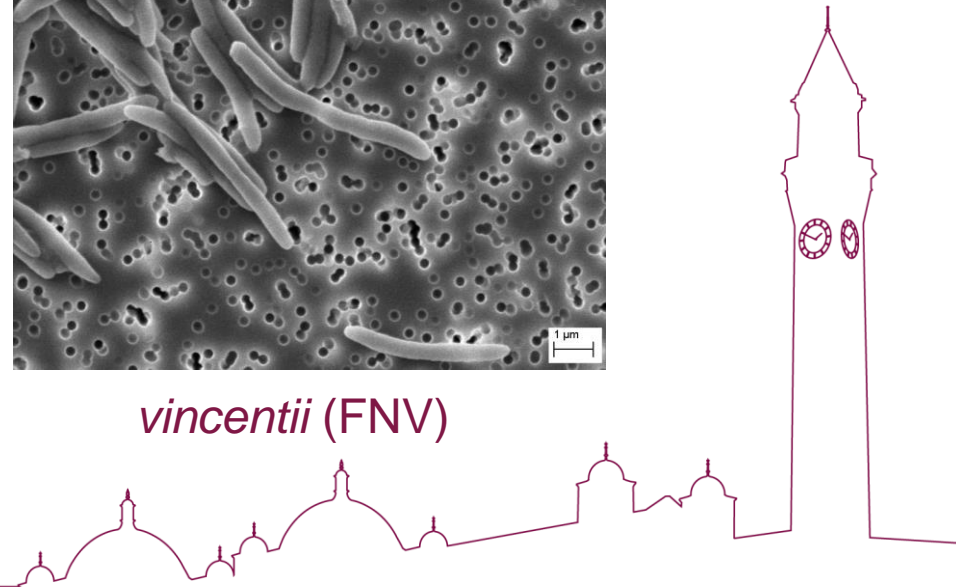
nucleatum (FNN)



polymorphum (FNP)



vincentii (FNV)



Differences among subspecies

- Involvement in health and disease
 - FNF and FNV isolated from healthy sites^{1,7}
 - FNA and FNN from diseased sites⁷⁻⁹
 - FNP associated with both⁸
- Subspecies-specific host response modulation in HL-60 cells¹⁰
- Differential incorporation of subspecies into multispecies biofilm models¹¹



Is there a difference in single-subspecies biofilm formation?

...but *F. nucleatum* does not form biofilms on its own *in vivo*...

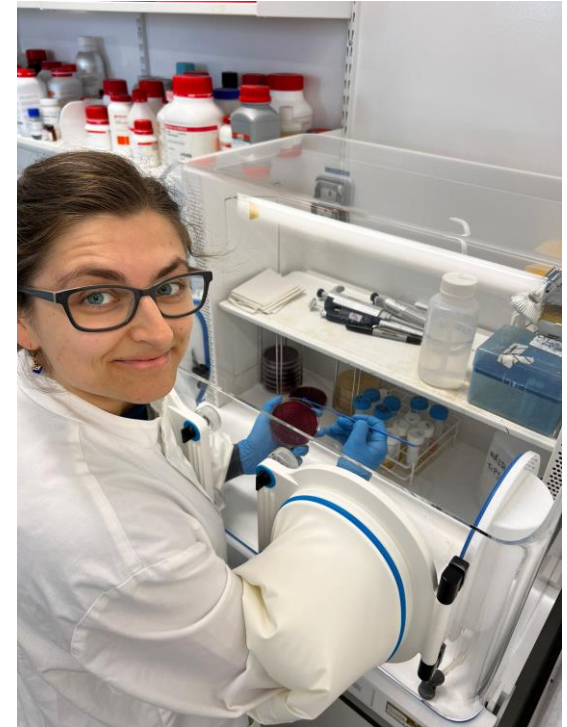
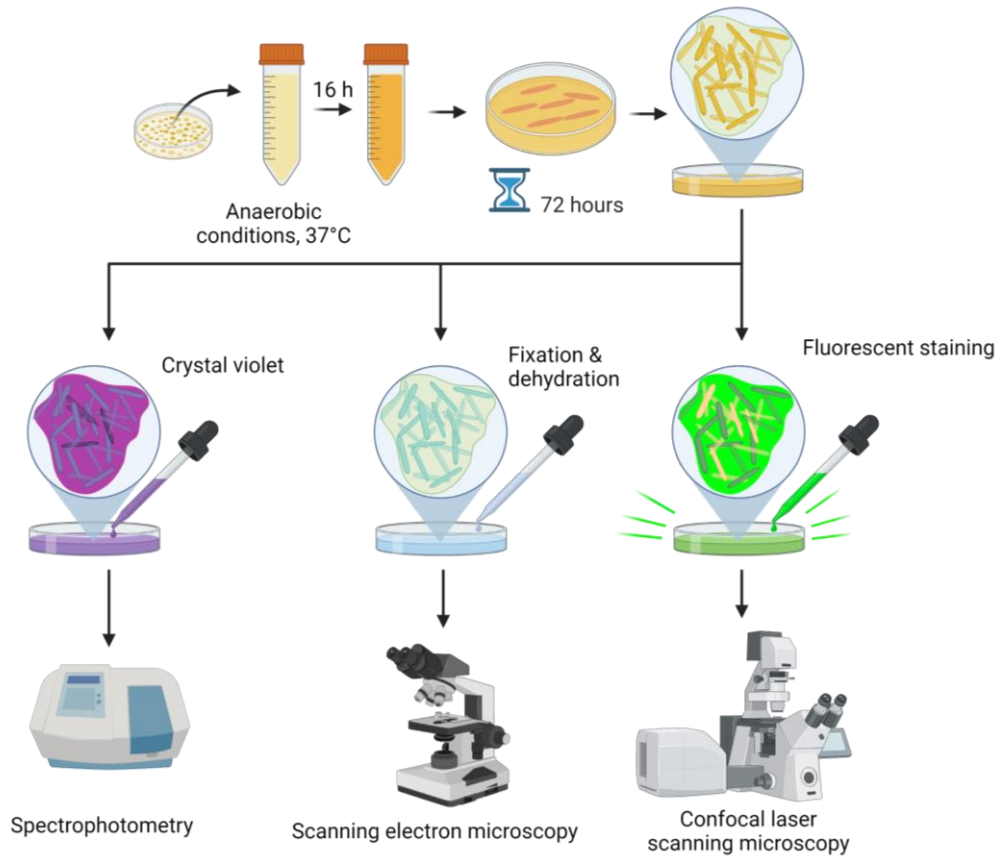
That is true, but...

- **Absence of binding partners allows better understanding of biofilm-related immunogenic and pathogenic properties and expression of virulence factors**
- **Using a simplified single-subspecies biofilm model which can be repeated by other researchers**



Methodology

In vitro



Biofilm thickness and stability varies among *F. nucleatum* subspecies and on different surfaces

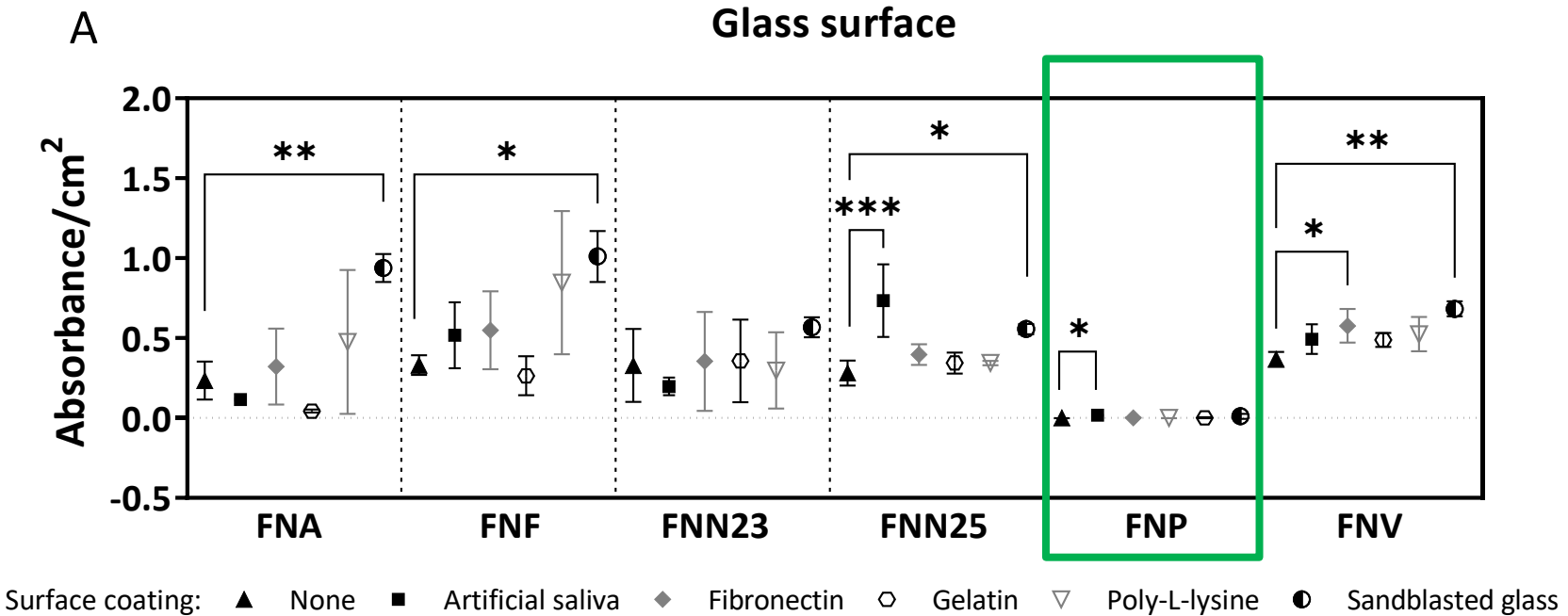


Figure 1. Single-subspecies biofilms quantified using CV. A: Quantification on glass coverslips with or without (control) surface coatings. One-way ANOVA was performed followed by Dunnett's post hoc test for within subspecies differences between control (uncoated glass) and test samples (*= $p < 0.05$; **= $p < 0.01$; ***= $p < 0.001$), $n=3$.

Biofilm thickness and stability varies among *F. nucleatum* subspecies and on different surfaces

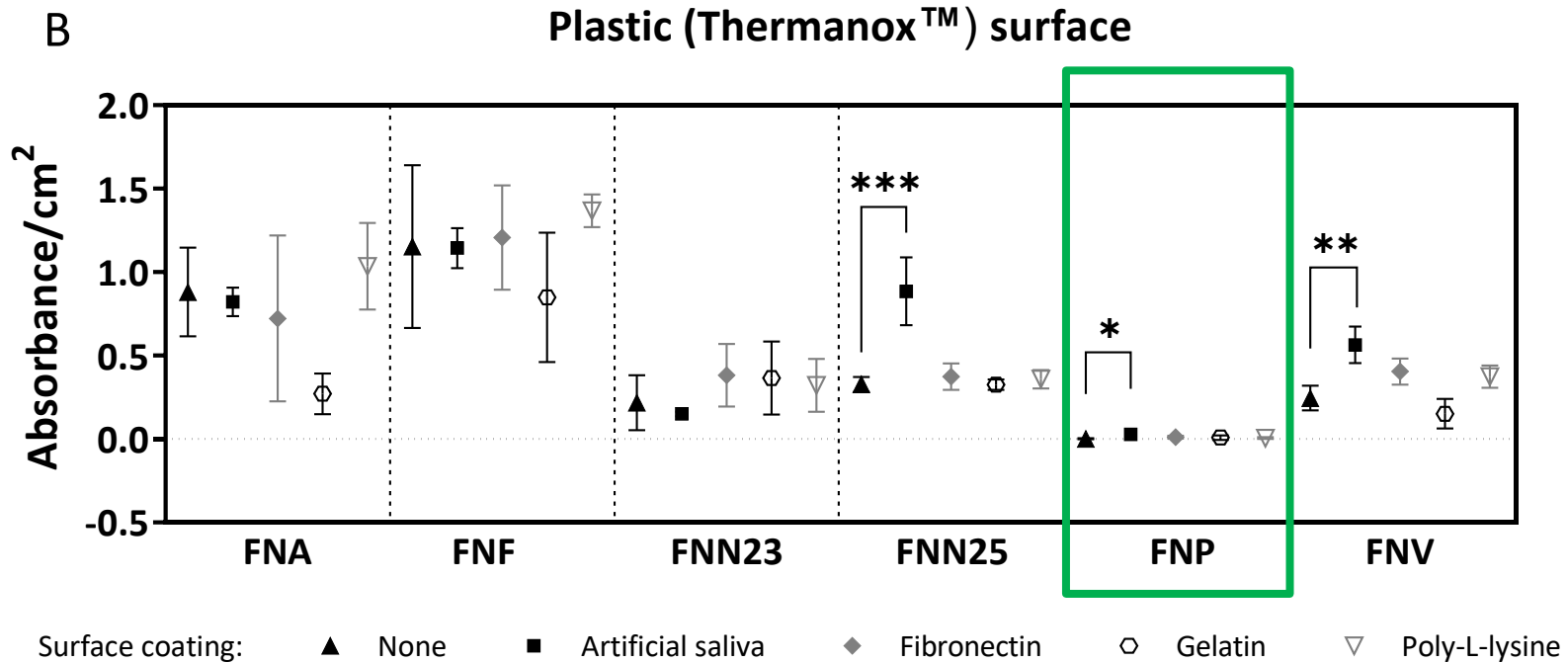
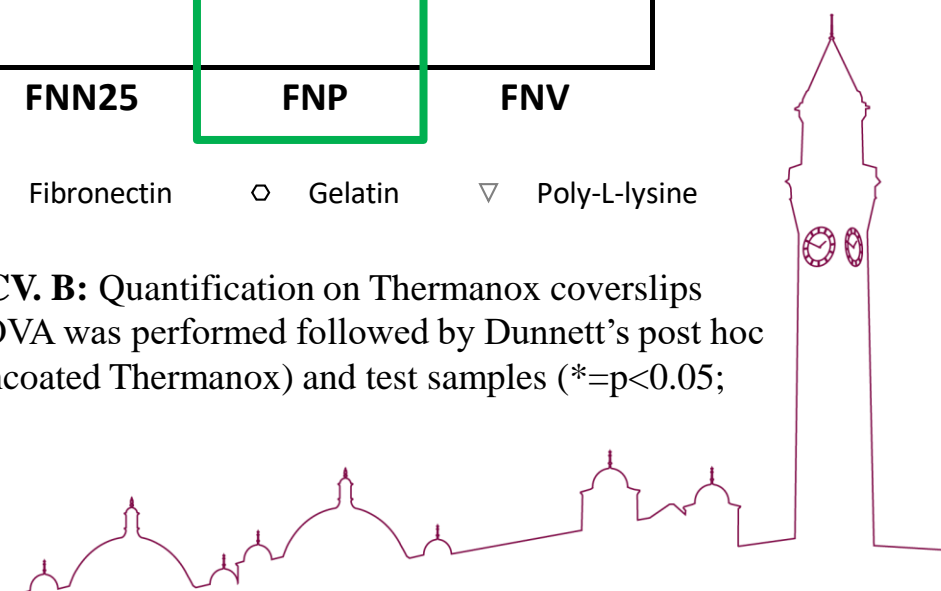


Figure 1. Single-subspecies biofilms quantified using CV. B: Quantification on Thermanox coverslips with or without (control) surface coatings. One-way ANOVA was performed followed by Dunnett's post hoc test for within subspecies differences between control (uncoated Thermanox) and test samples (*= $p < 0.05$; **= $p < 0.01$; ***= $p < 0.001$), $n=3$.



Biofilm architecture differs among *F. nucleatum* subspecies

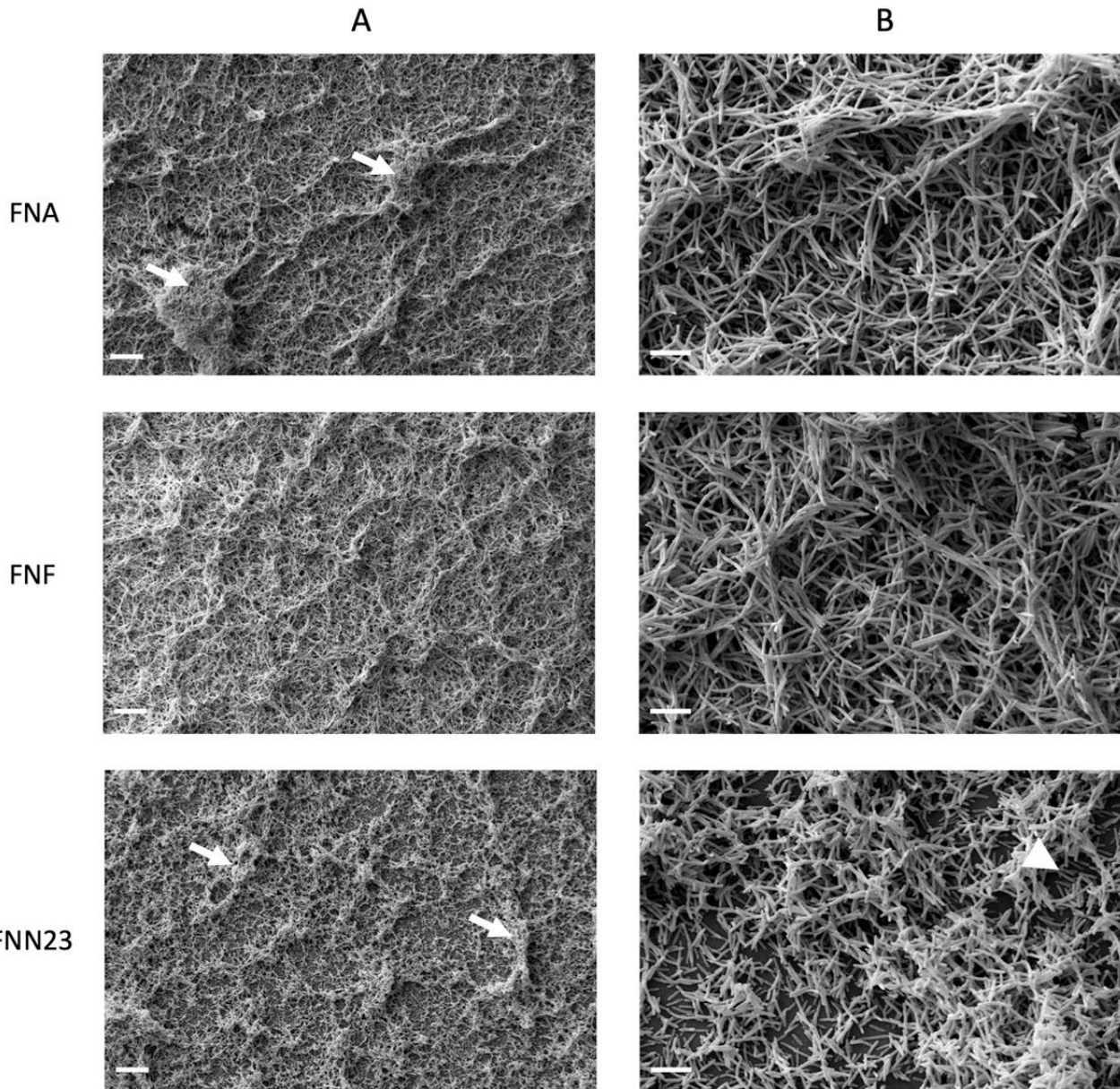


Figure 3. *F. nucleatum* biofilms grown on poly-L-lysine coated Thermanox coverslips. A: 1000X magnification, scale bar 20µm. **B:** 5000X magnification, scale bar 5µm. Biofilms from two independent experiments grown in duplicates were imaged and representative micrographs are shown.

Biofilm architecture differs among *F. nucleatum* subspecies

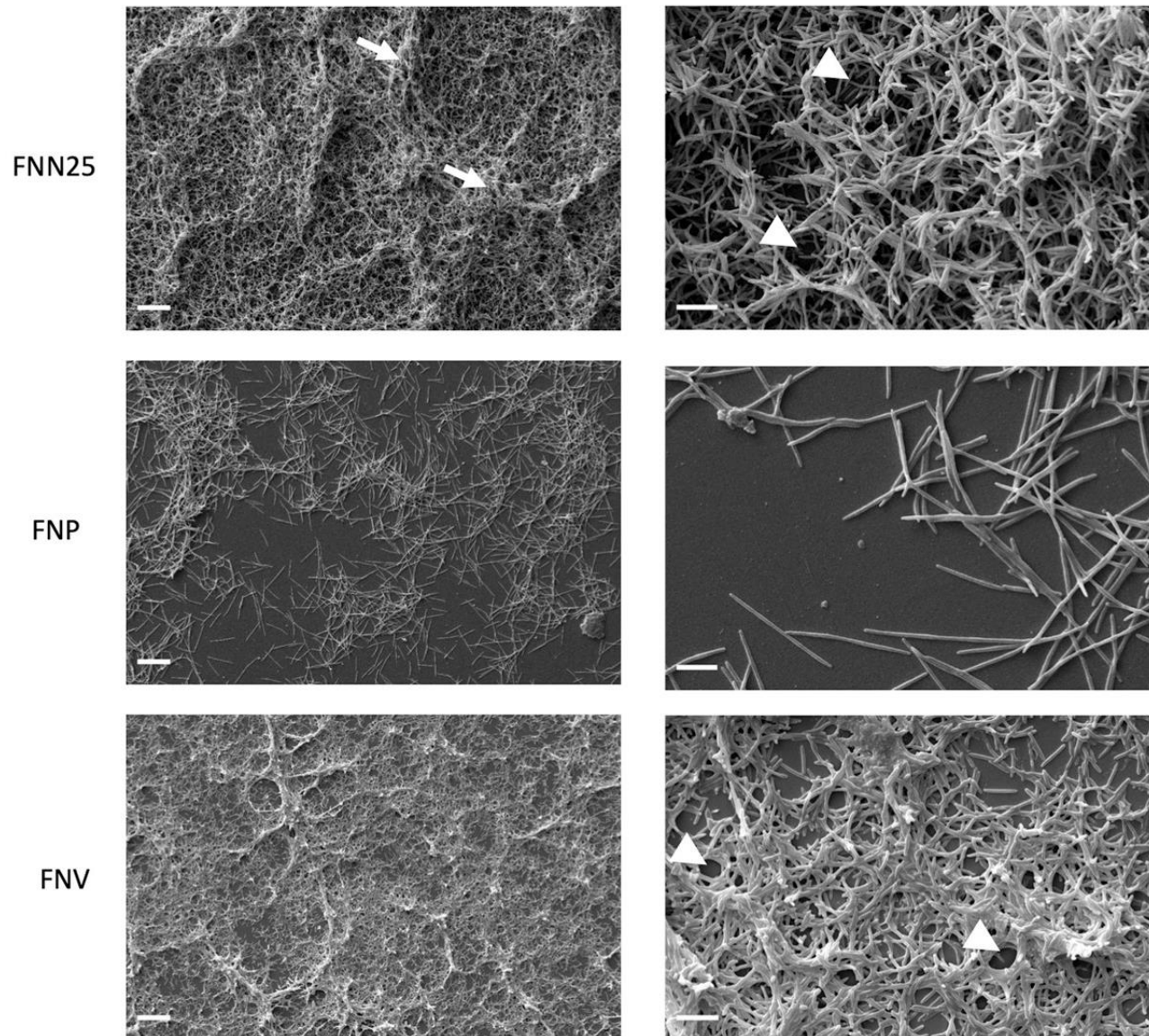
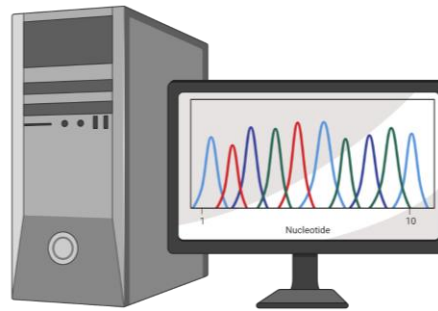


Figure 3. *F. nucleatum* biofilms grown on poly-L-lysine coated Thermanox coverslips. **A:** 1000X magnification, scale bar 20 μ m. **B:** 5000X magnification, scale bar 5 μ m. Biofilms from two independent experiments grown in duplicates were imaged and representative micrographs are shown.

Why does FNP differ from other subspecies?



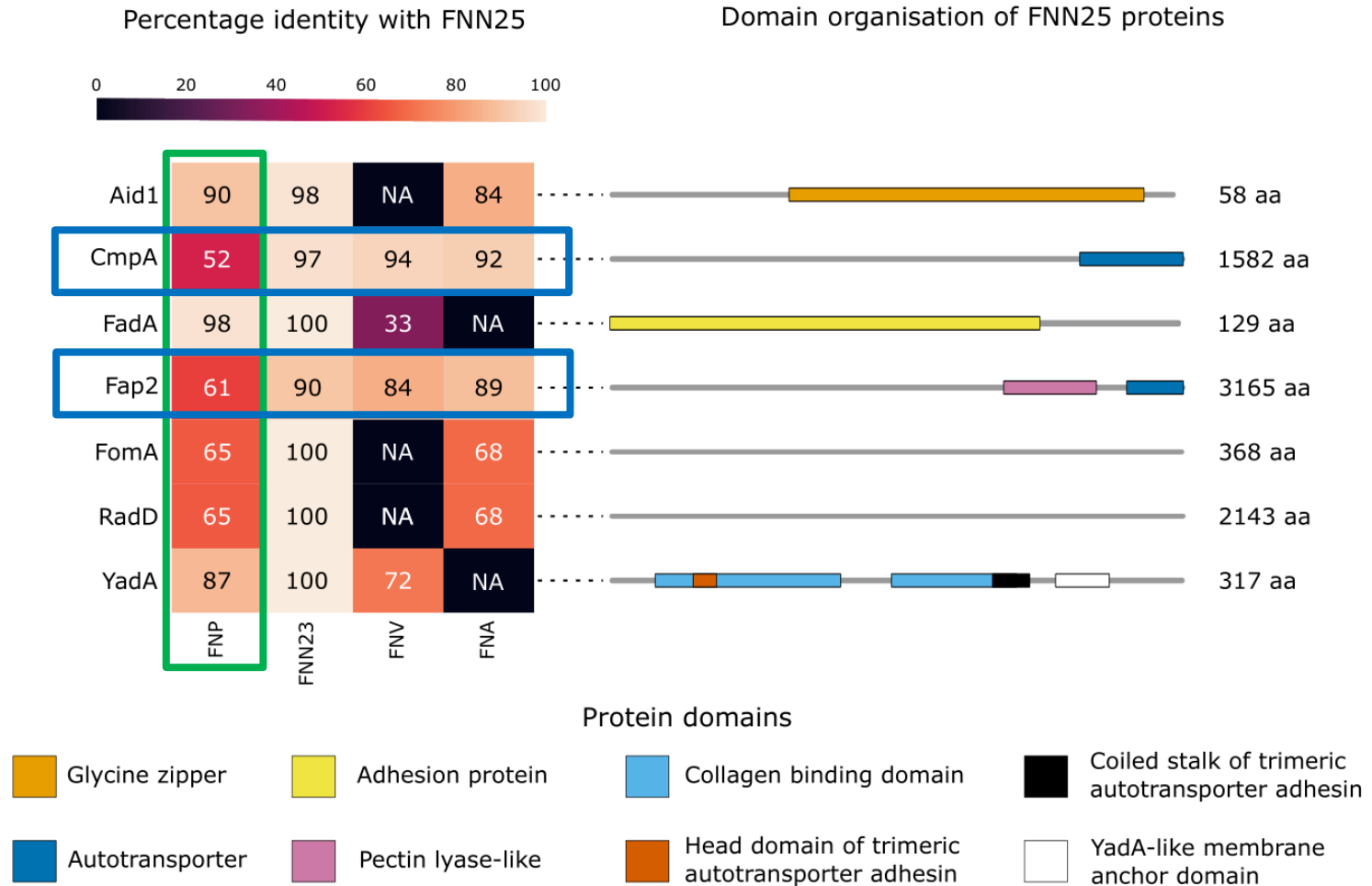
Bioinformatics



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Conservation of adhesion protein orthologues varies among *F. nucleatum* subspecies



Conservation of adhesion protein orthologues varies among *F. nucleatum* subspecies

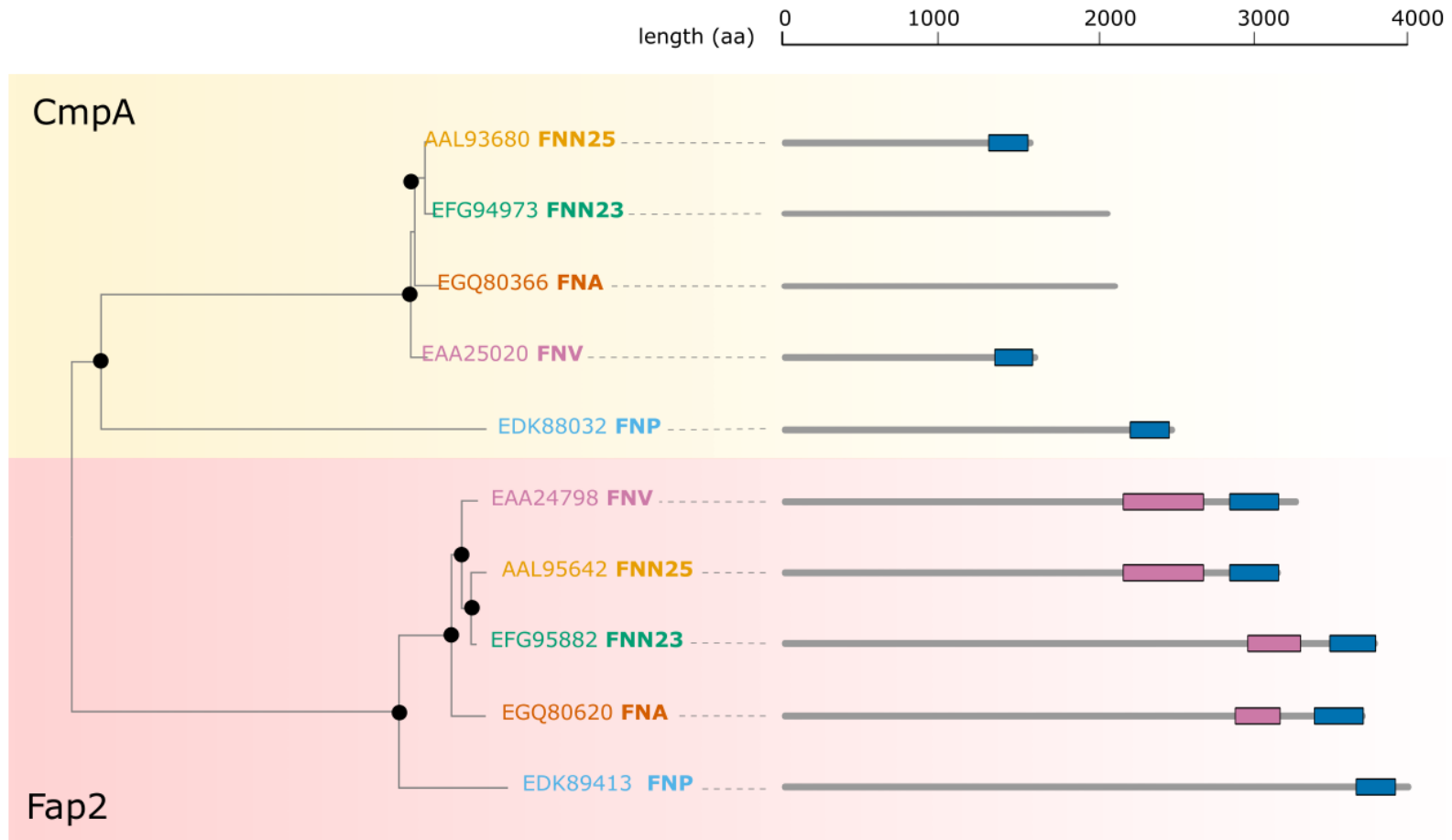
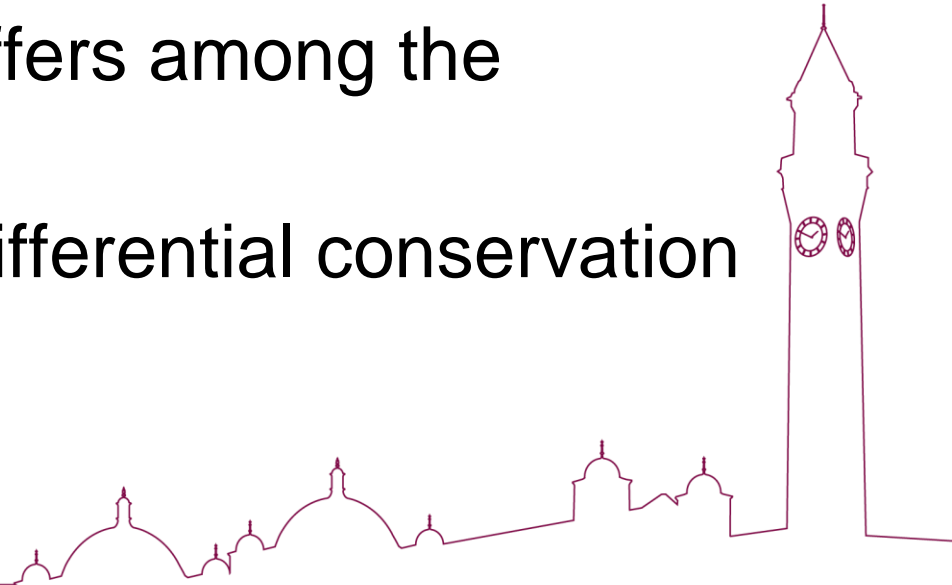


Figure 4. Bioinformatic analysis of adhesion proteins in *F. nucleatum* subspecies. CmpA and Fap2 phylogenetic tree with black circles representing bootstrap values > 95. Autotransporter domains are coloured in blue, pectin lyase-like domains in pink.

Conclusions

- In our experimental conditions, *F. nucleatum* subspecies differ in biofilm thickness and stability
- FNP does not form stable biofilms
- Formation of biofilms is substrate-specific
- Architecture of biofilms differs among the subspecies
- Adhesion proteins have differential conservation *in silico*



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