

INTRODUCTION

The sticky mucus in airways of CF patients favors the colonization by bacteria [1] that tend to form difficult to-treat biofilms [2]. Although *S. aureus* (SA) and *P. aeruginosa* (PA) can be isolated concomitantly in the sputum of these patients, in-vitro biofilm models with reference strains often claim that PA overgrows SA [3].

ASM: mimics the viscoelastic properties of the mucus found in the airways of CF patients [5]

✓ higher elasticity than viscosity

✓ Composition (per liter):

10 g mucin; 4 g DNA; , 5.9 mg DTPA ; 5 g NaCl; 2.2 g KCl; 3 g agar; 5 g amino acids; 1.81 g Tris; 5 ml egg yolk emulsion

OBJECT

- Establish dual-species biofilms using artificial sputum (ASM)
- Characterise the resulting biofilms for different phenotypes of PA

METHODS

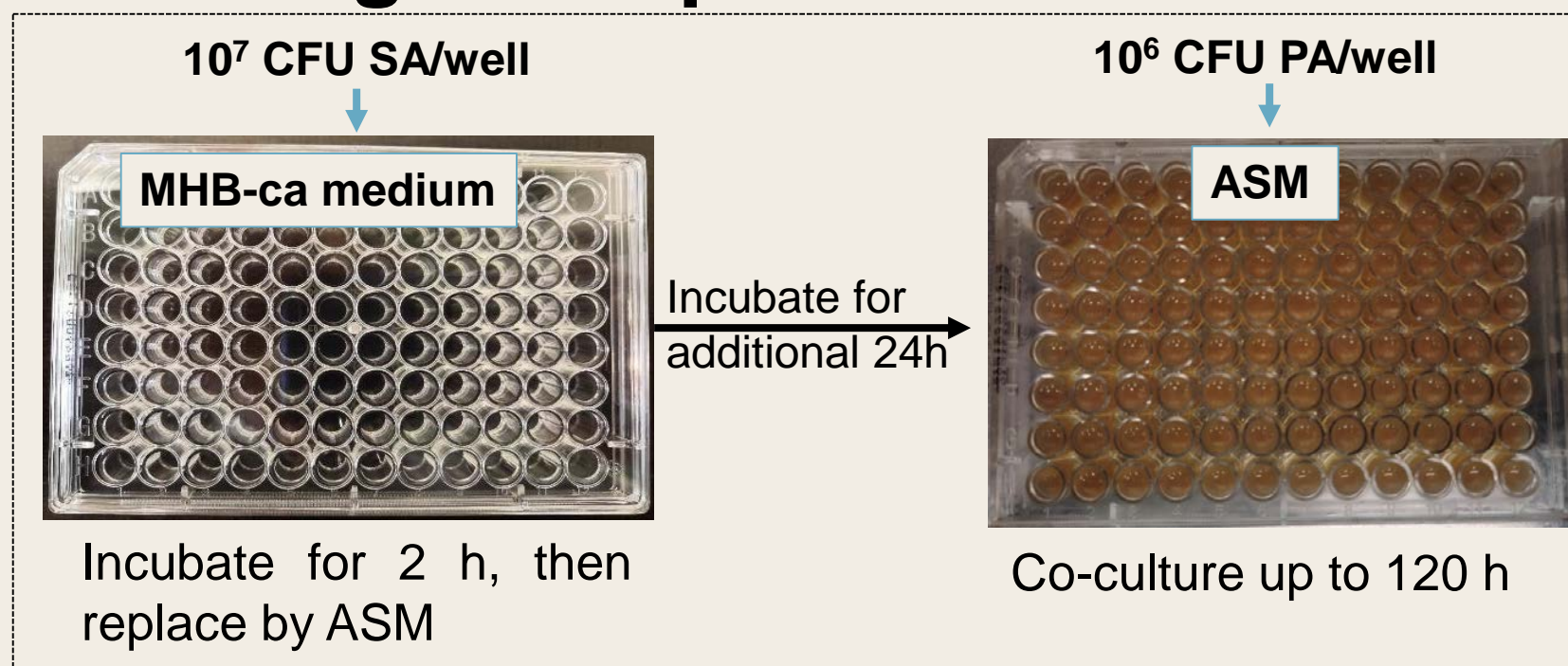
classification of PA



all SA → same phenotype

- The distribution of PA and SA was observed in confocal microscopy after fluorescence in situ hybridization

biofilm growth process



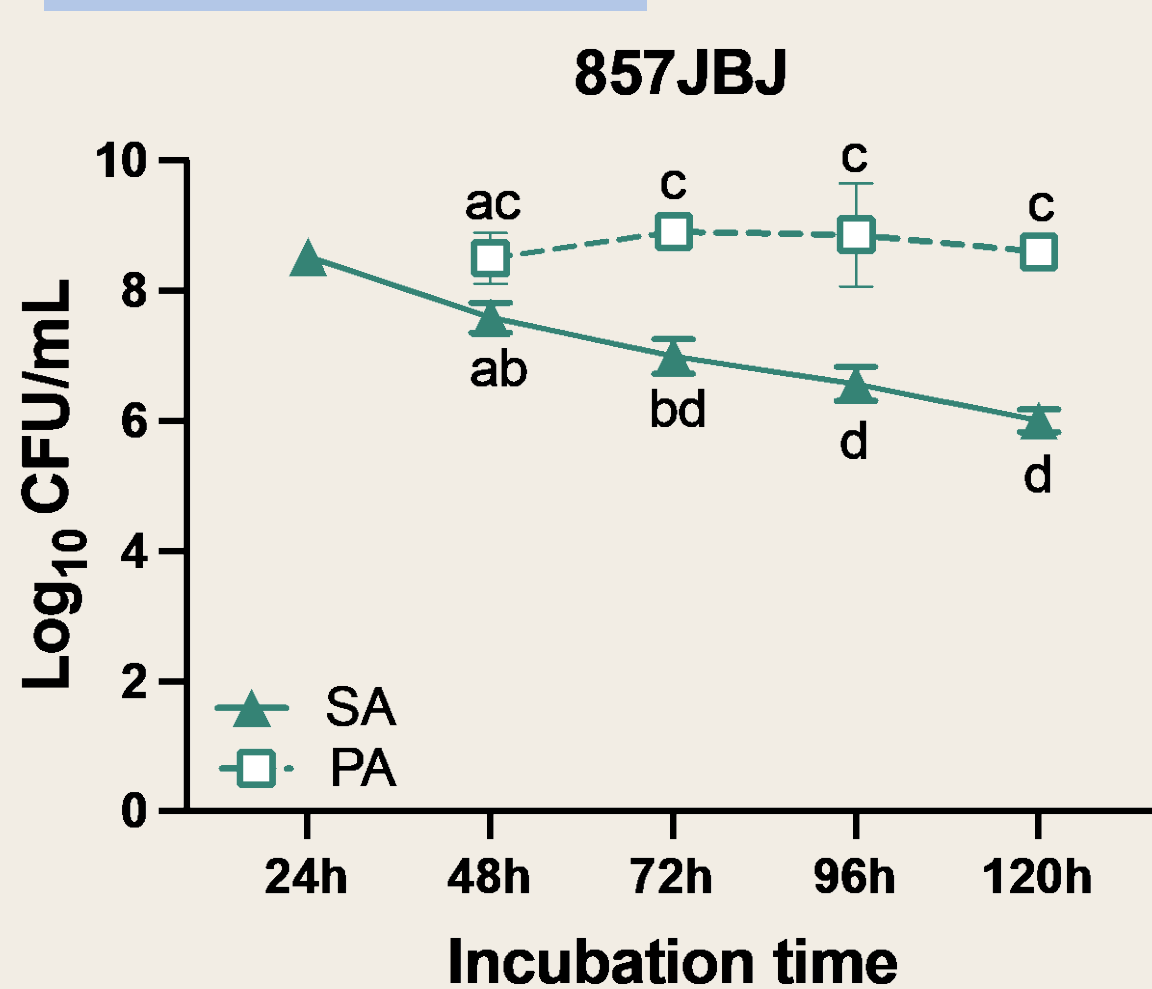
characterization of biofilms

- The biomass was quantified by crystal violet staining
- CFUs were counted on species-specific agar media

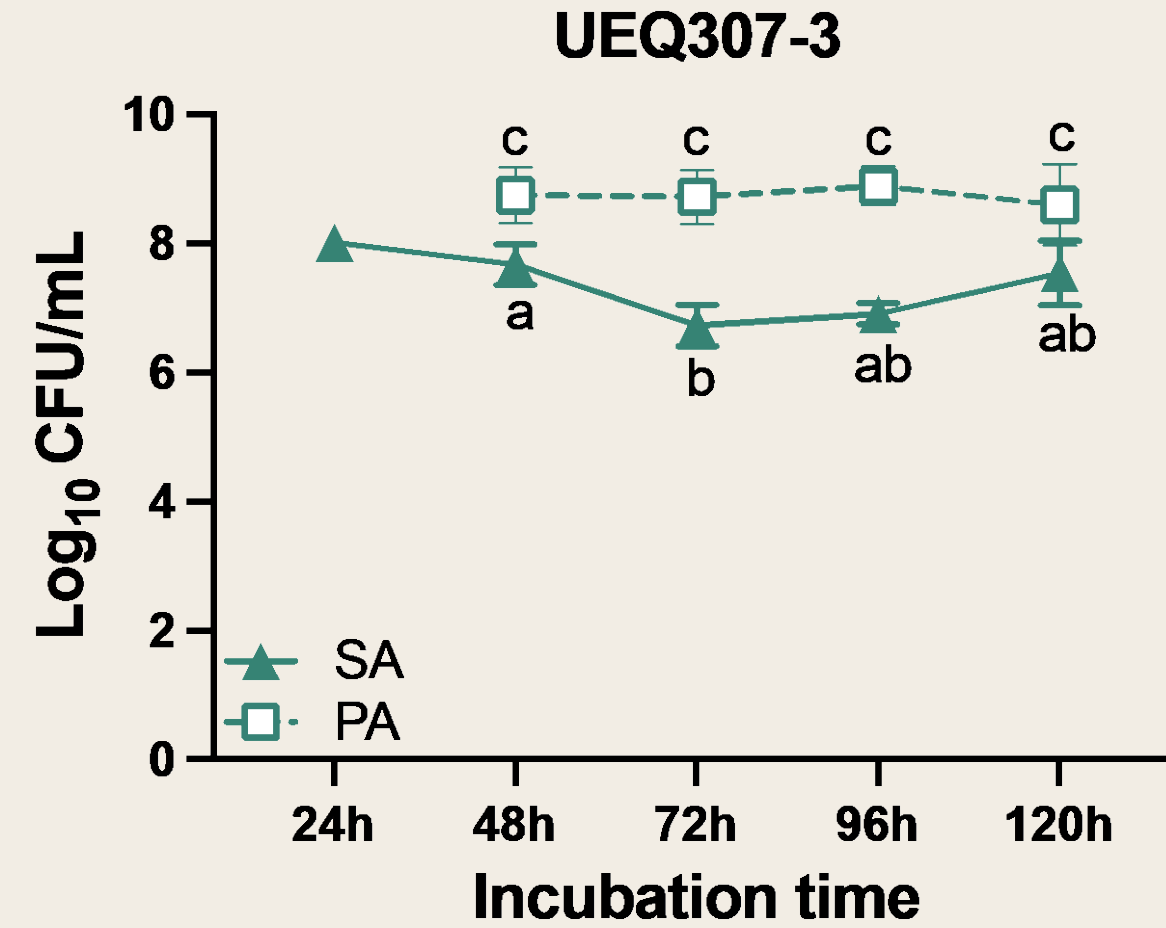
RESULTS AND CONCLUSION

- SA remained steady in all dual-species biofilms over time (mucoid or SCV PA) or after 72 h (non mucoid PA).
- PA was stable over time (non-mucoid or SCV) or after 72h (mucoid)
- Biomass was stable over time for all biofilms

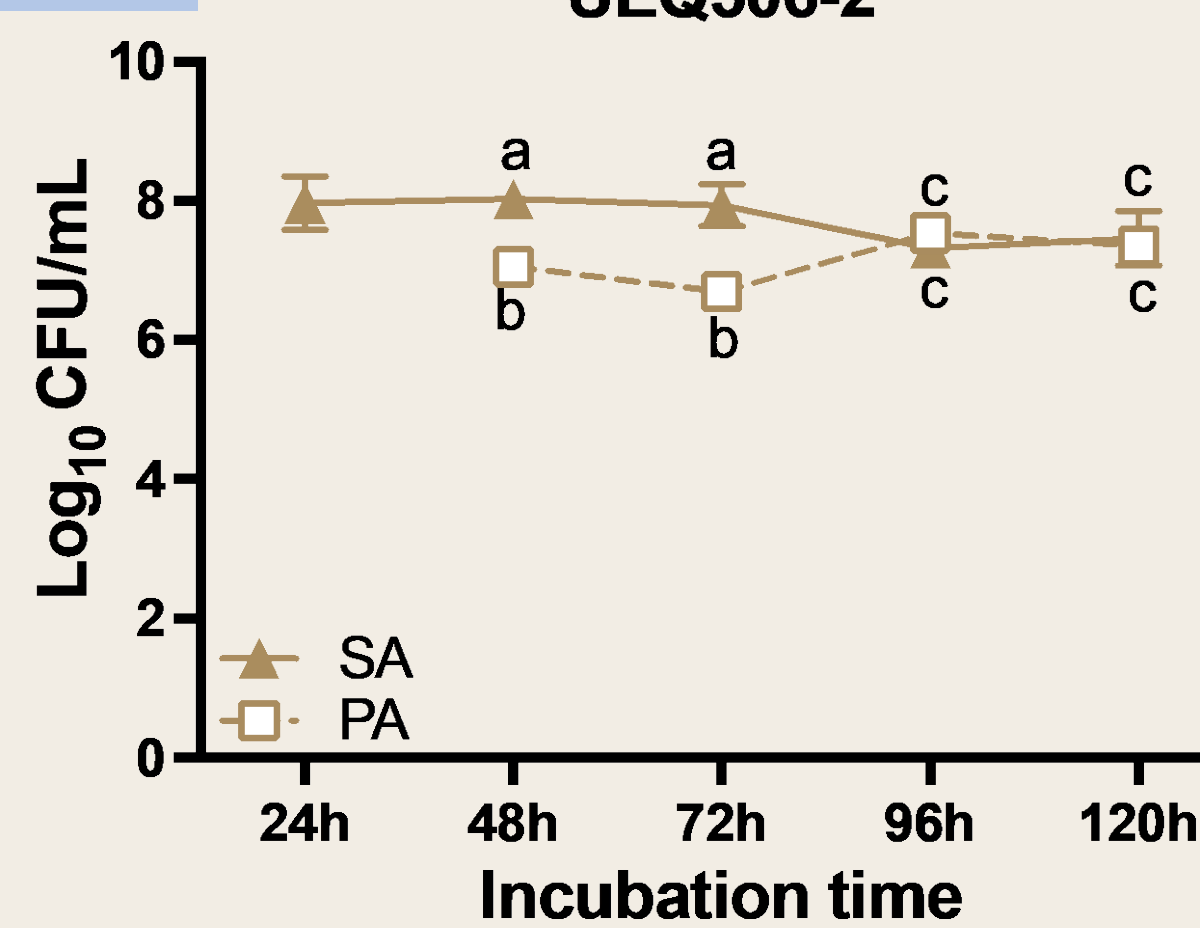
A: non-mucoid



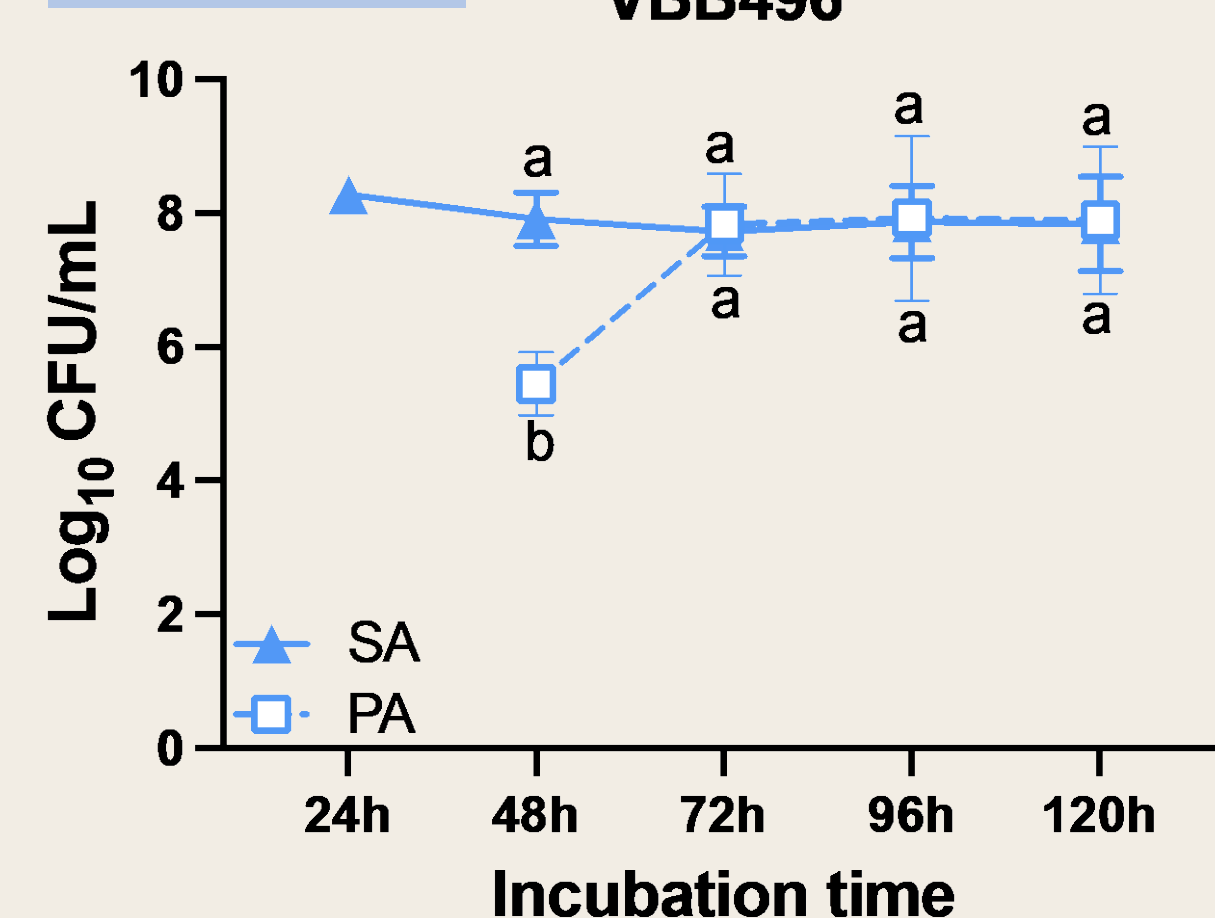
C: SCV



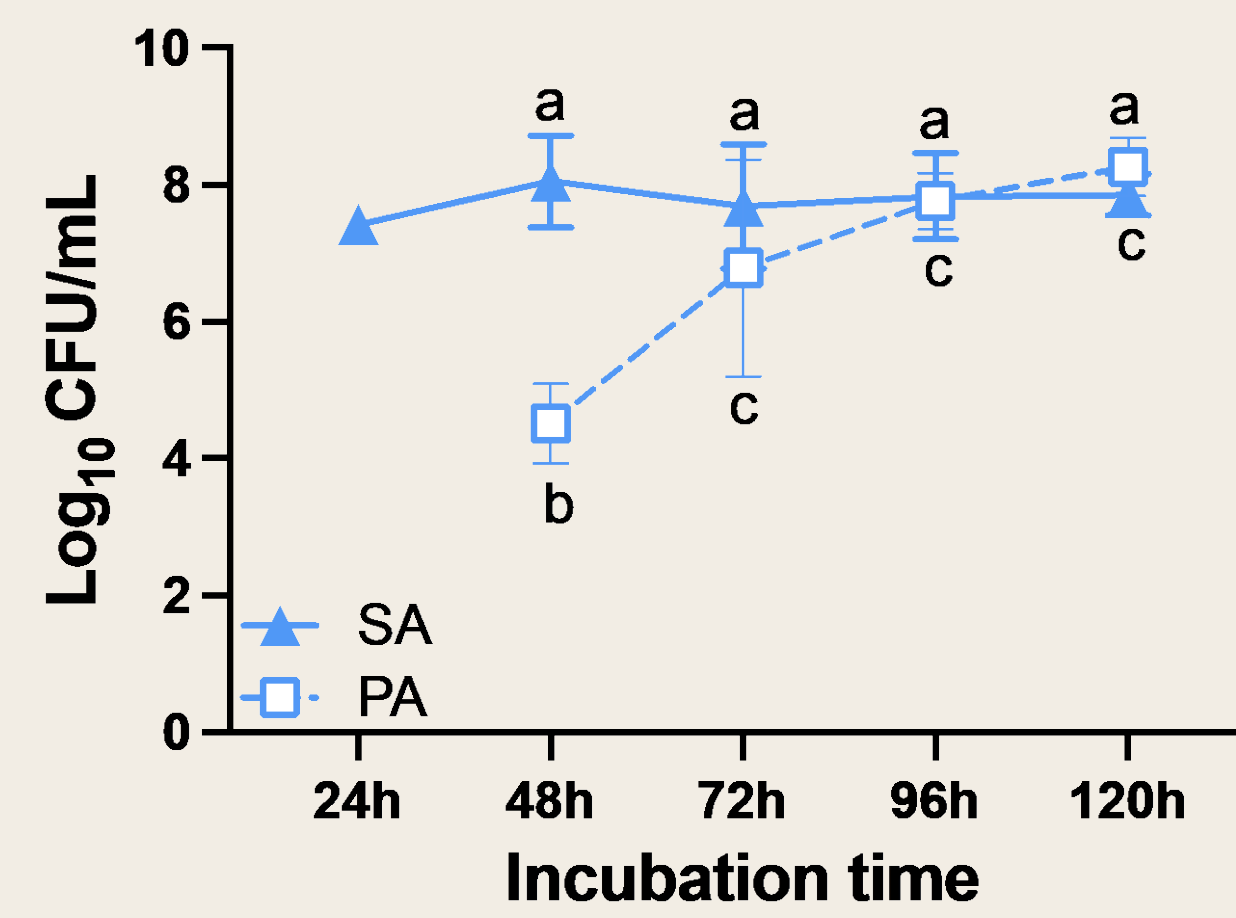
UEQ306-2



B: mucoid



492IVJ



D: biomass

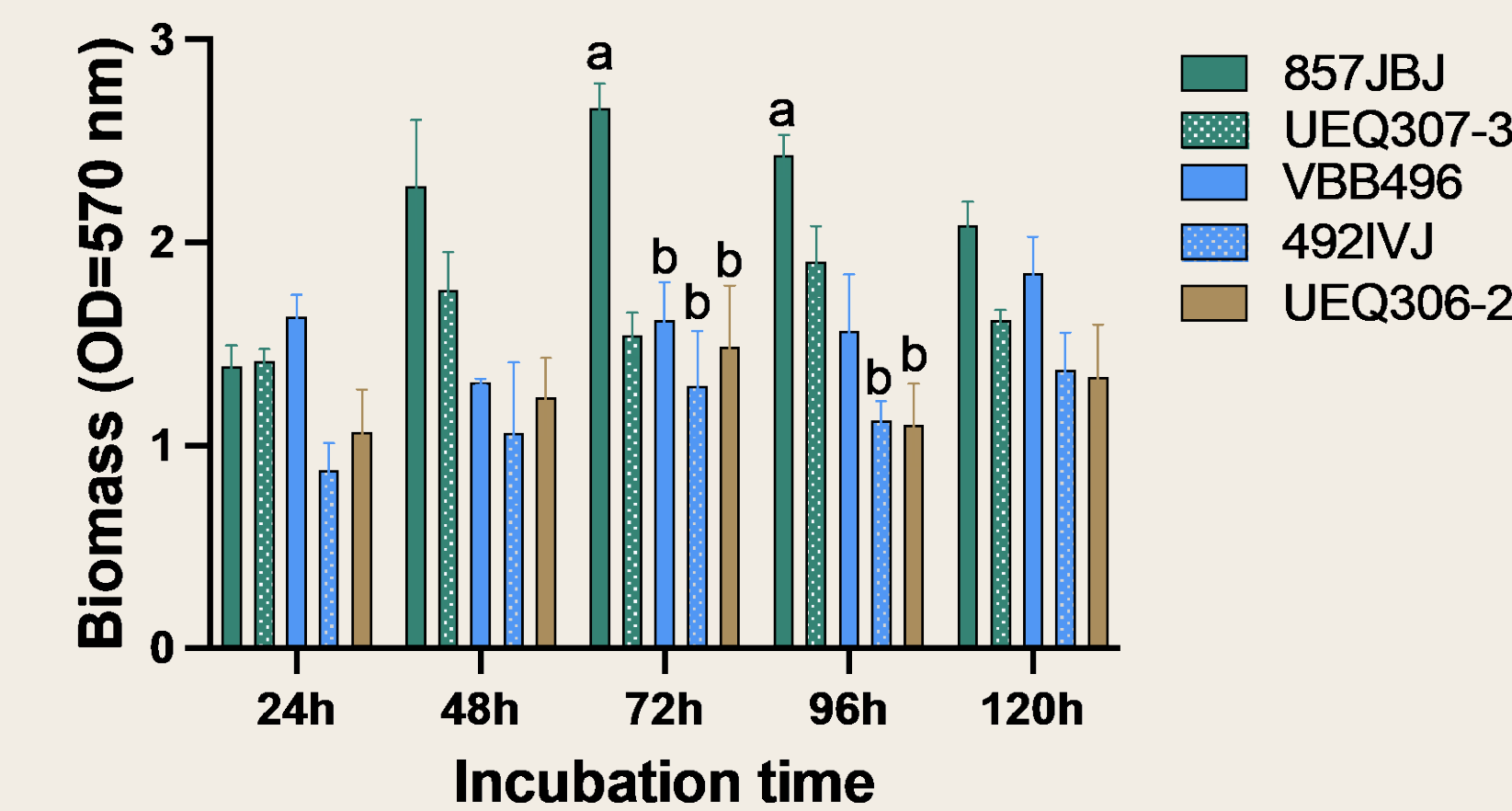


Fig. 1. Evolution over time of CFUs (A-C) or biomass (D) in dual species biofilms of clinical isolates of PA and SA for different phenotypes of PA (A: non-mucoid; B: mucoid; C: SCV). Values are means ± SEM from 3 or 4 independent experiments performed in more than triplicates; values with different letters are significantly different from each other (P<0.05). 2-way ANOVA followed by Tukey's multiple comparisons test of pairs of isolates. In panel D, statistics are indicated only for time points for which some differences are seen.

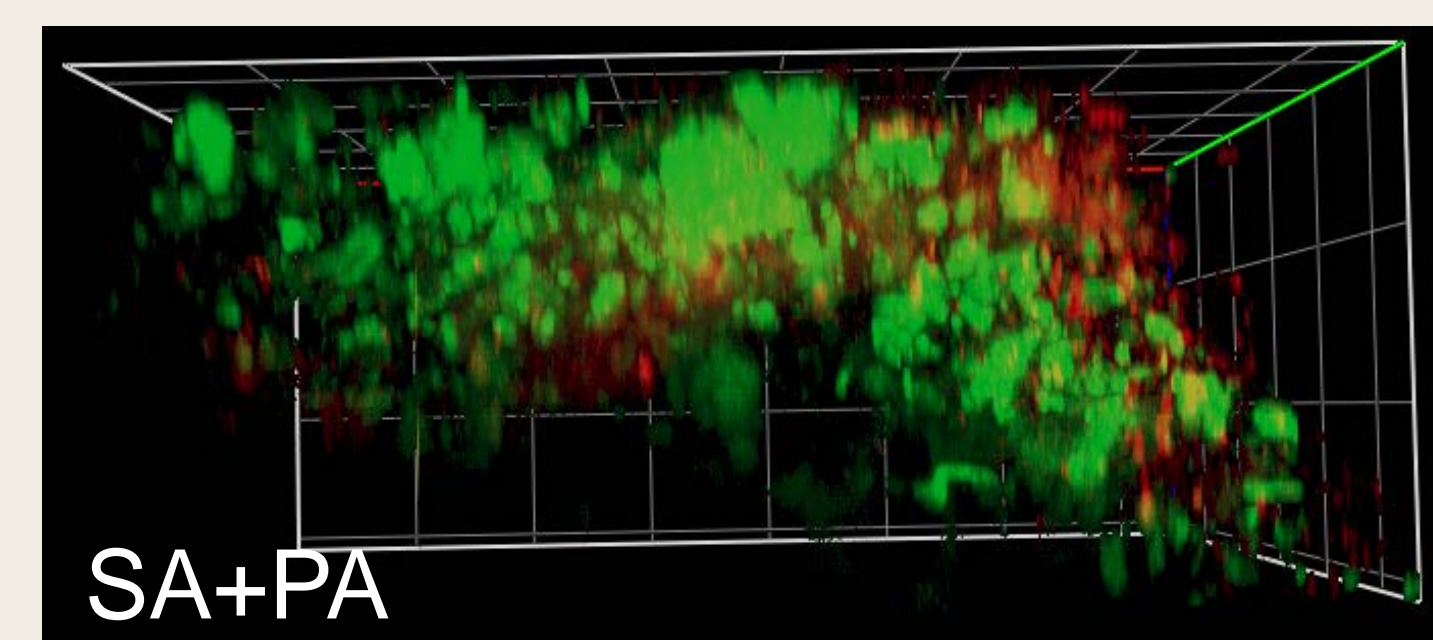
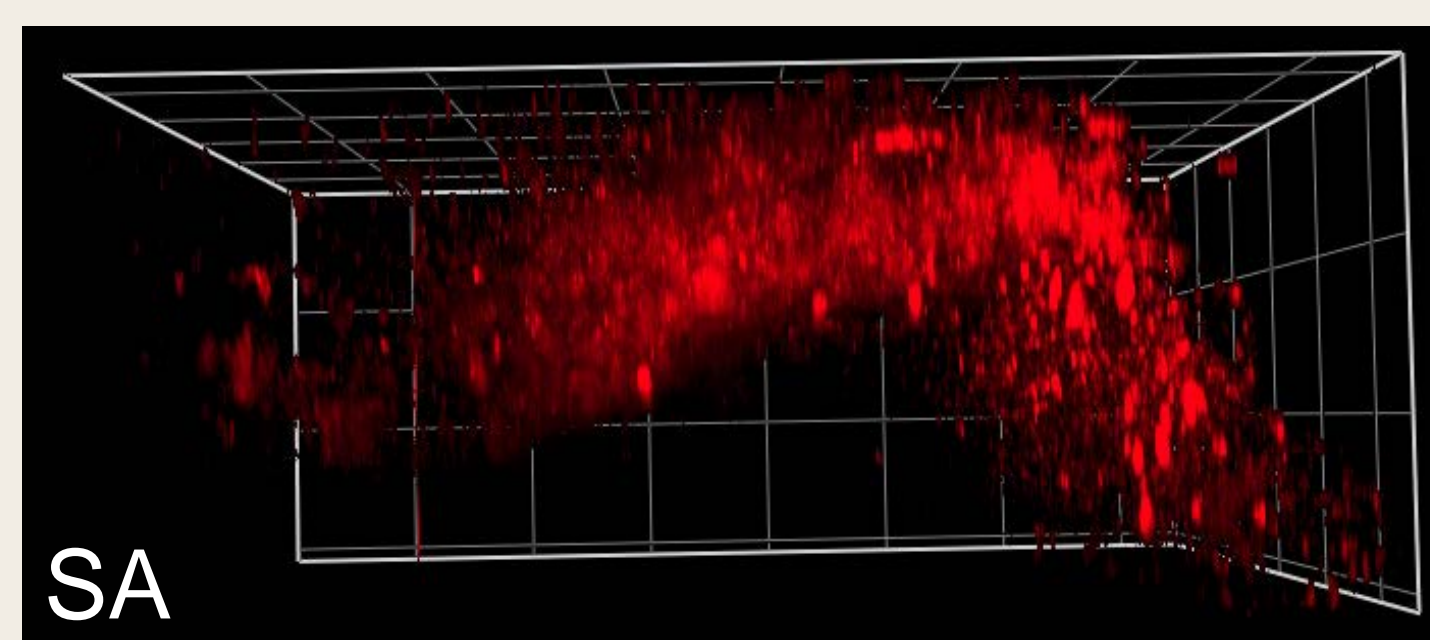
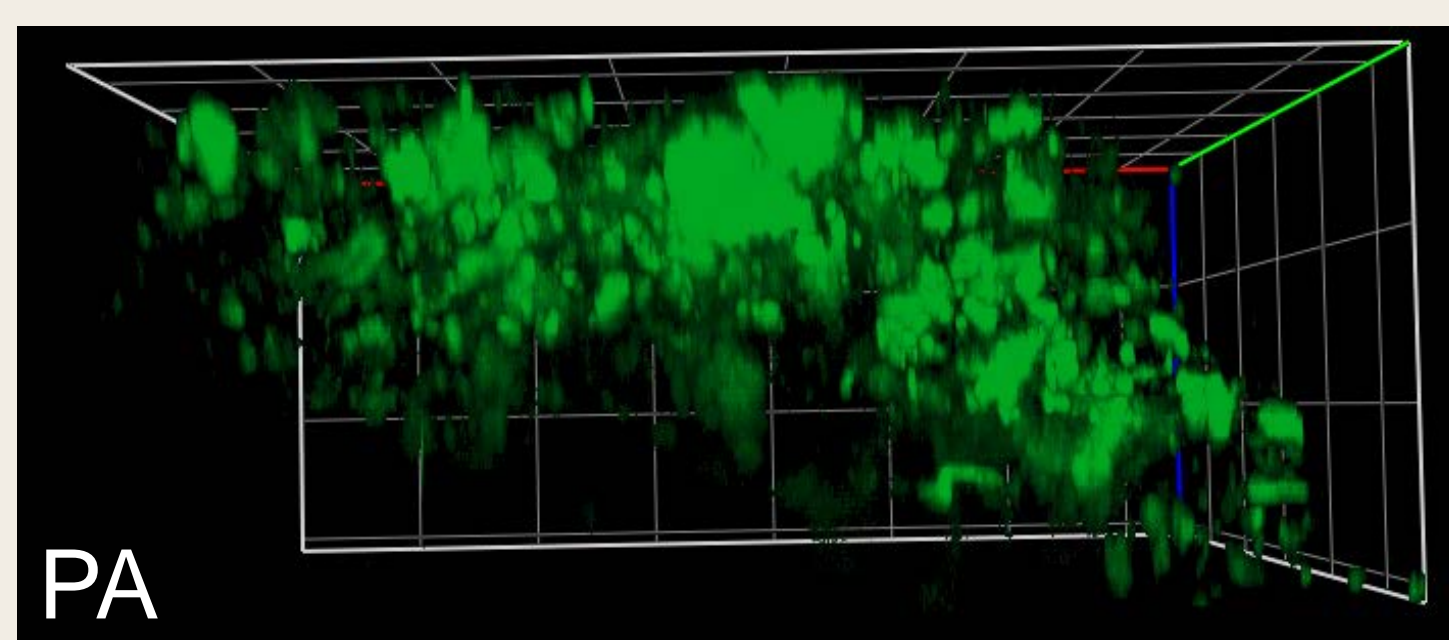


Fig. 2. illustrates for a representative pair of isolates with mucoid PA (VBB496) that SA and PA aggregated with each other in biofilms.

- Dual-species biofilms easily form in ASM with isolates obtained from the same patients, and SA is not outcompeted by PA, whatever its phenotype. Furthermore, SA and PA can grow in common aggregates. This is consistent with the cross-adaptation of PA and SA observed for isolates collected after several years in CF patients [4].

REFERENCES

- Ratjen et al. 2015 Nat Rev Dis Primers 1: 15010.
- Høiby et al. 2015 Clin Microbiol Infect 21: S1-S25.
- Filkins et al. 2015 J Bacteriol 197(14):2252-64.
- Baldan et al. 2014 PLoS One 9 (3): e89614.
- Diaz Iglesias et al. 2019 Antimicrob Agents Chemother 63: e02204-19

Acknowledgements:



A copy of this poster will be made available after the meeting at <http://www.facm.ucl.ac.be/posters.htm>