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Introduction

- S. aureus* (Sa) and *P. aeruginosa* (Pa) cause difficult-to-treat infections in patients with cystic fibrosis (CF), related to their capacity to form biofilms in the thick mucus obstructing their airways. Sa is most frequent in children and Pa, in adults. Yet both species can also be co-isolated from a single patient. However, in-vitro biofilm models with reference strains often claim that Pa overgrows Sa in dual-species biofilms (1).

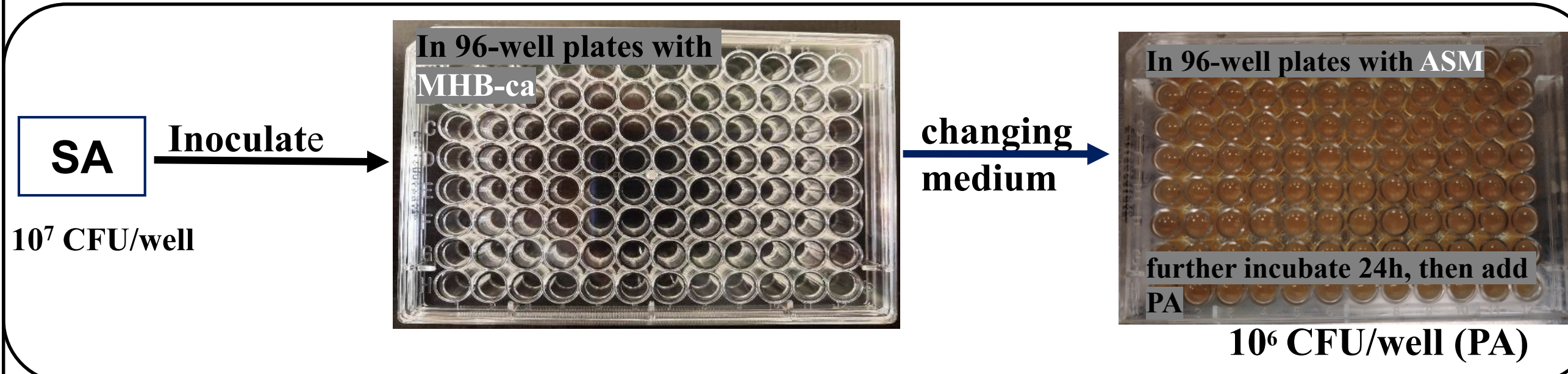
Aim of the study

- To develop a dual-species biofilm of Sa and Pa growing in artificial sputum medium (ASM) showing viscoelastic properties similar to those of the mucus of CF patients (2), comparing reference strains with clinical isolates concomitantly collected from the same patient.

Methods

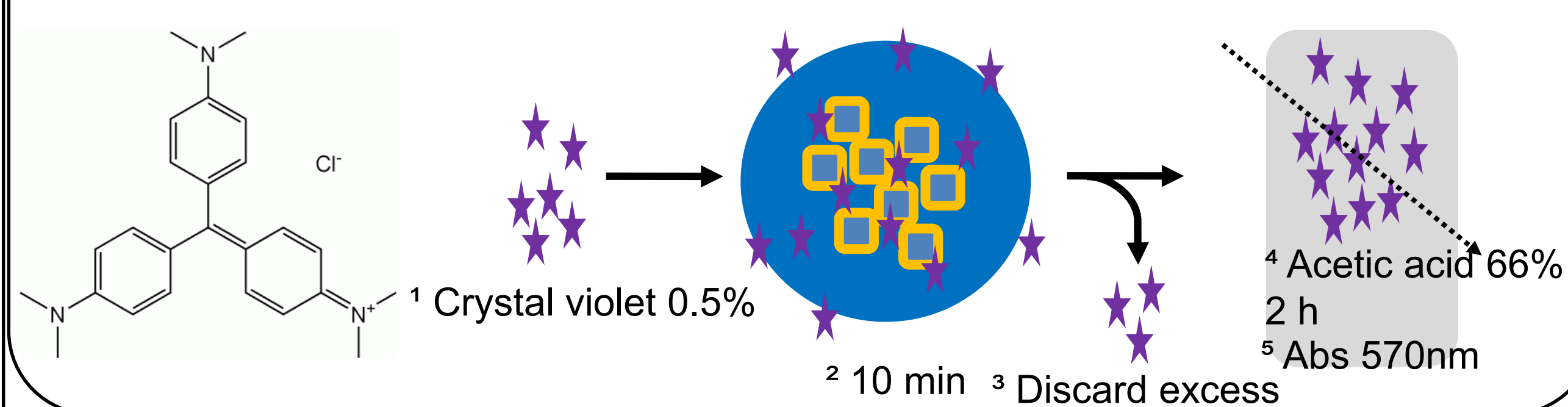
- Dual species biofilms were developed using reference strains SaATCC25923 (SaATCC) and PAO1, or a pair of clinical co-isolates Sa-PaVBB496 (Sa-PaVBB) (paired cultures), or cross-cultures (i.e., SaATCC+PaVBB, or PAO1+SaVBB) in ASM.

Biofilm growth process

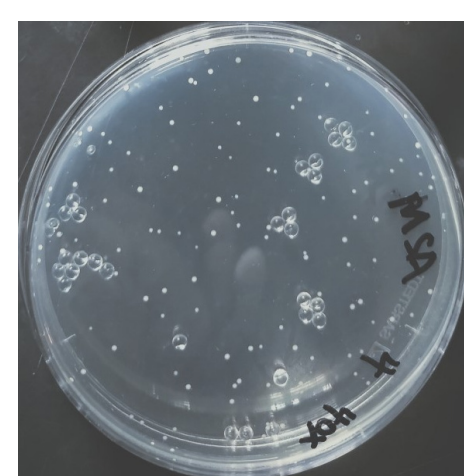


characterization of biofilms

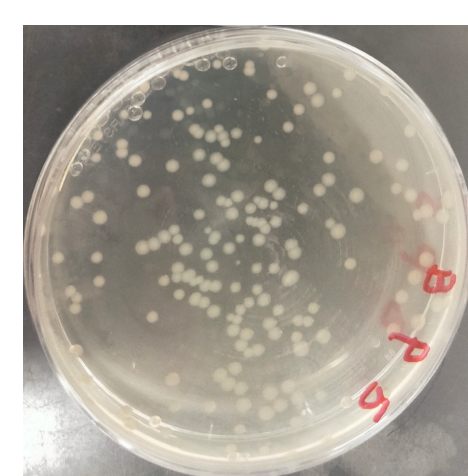
crystal violet staining: matrix production



colony counts (Selective agar plates): microorganism viability



Mannitol Salt Agar (SA)



Pseudomonas Isolation Agar (PA)

ASM composition

10 g mucin, 4 g DNA, 5 g NaCl, 2.2 g KCl, 3 g agar, 5.9 mg DTPA (diethylenetriaminepentaacetic acid), 5 g amino acids, 1.81 g Tris, and 5 mL egg yolk emulsion (per liter).

Results

PAO1 grew similarly in coculture with SaATCC or SaVBB, and expressed to similar levels QS genes in both cases. SaATCC CFUs remained stable in coculture with PaVBB but decreased in the presence of PAO1. PaVBB CFUs showed a profile similar to PAO1 in coculture with SaVBB but dramatically decreased over time when cocultured with SaATCC. PAO1 and PaVBB also harboured SCV phenotype. The biomass of this biofilm was lower than in other cases in spite of an overexpression of PaVBB QS genes in cross-cultures vs. paired cultures. SaVBB remained stable with both Pa. The Sa spa gene was the most upregulated in cross-cultures. sigB expression was undetectable in SaVBB.

Results

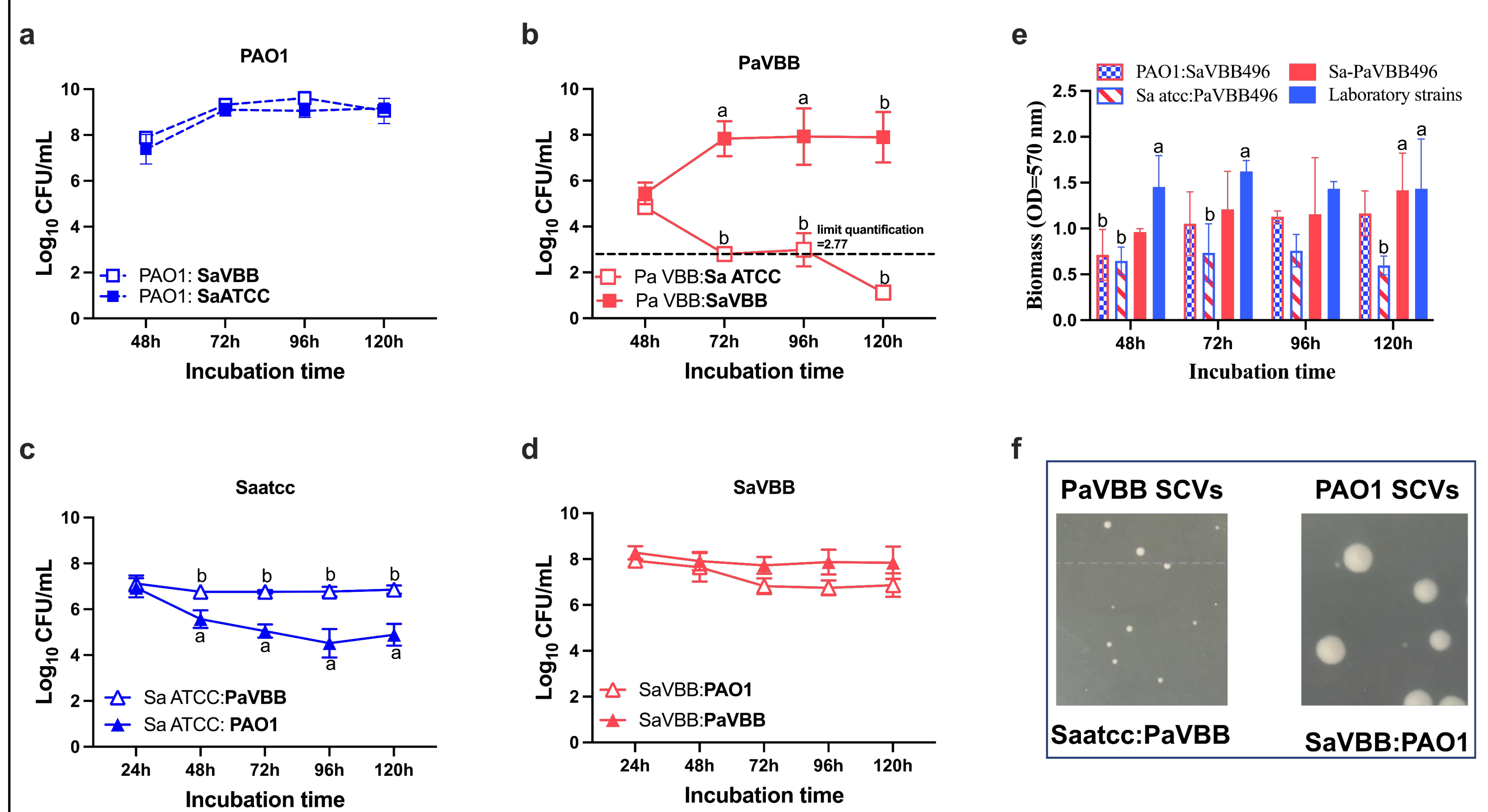


Fig. 1. Evolution over time of CFUs (a-d) or biomass (e) in dual species biofilm established by reference strains, a pair of clinic co-isolates or cross cultures (1 reference strain+1 clinical isolate of the other species.) Presence of small colony variants (SCVs) phenotype of PaVBB or PAO1 in cross-culture (f). Values are means \pm SEM from 3 or 4 independent experiments performed in more than triplicates; values with different letters are significantly different from each other ($P < 0.005$) (one-way ANOVA followed by Mann Whitney test).

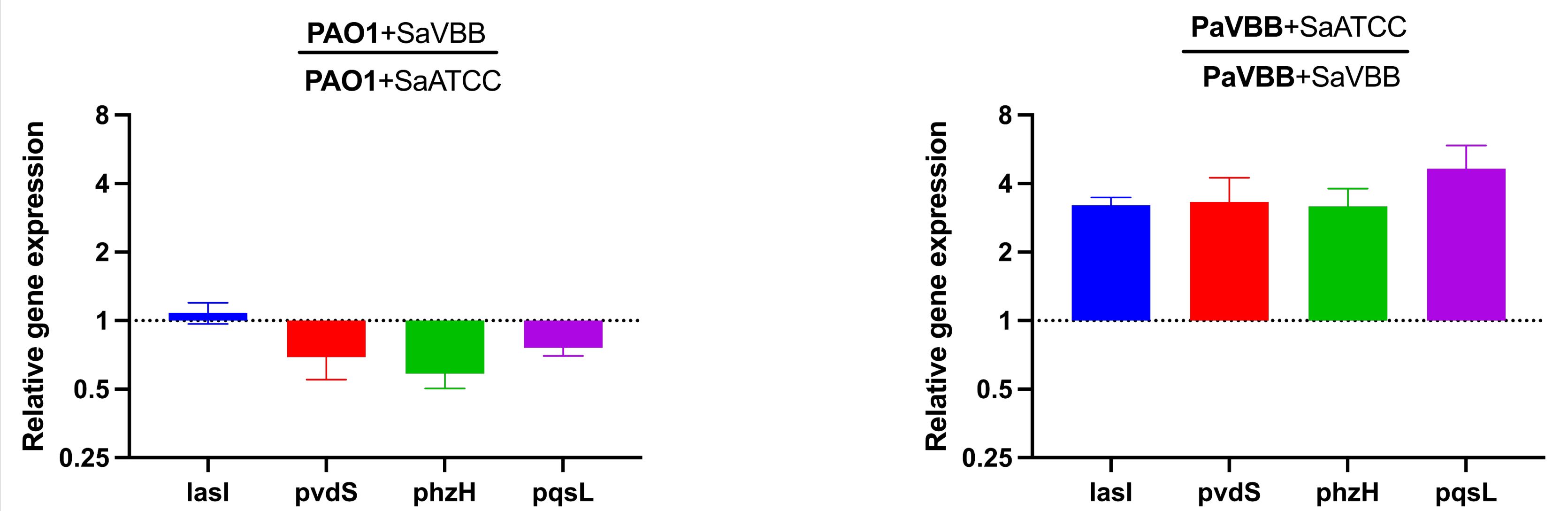


Fig. 2. *Pseudomonas aeruginosa* QS gene expression when co-cultured with reference strain SaATCC25923 or clinical isolate SaVBB at 72h. (A) relative expression of PAO1 QS gene in cross culture compared to reference biofilm (PAO1+SaVBB compared to PAO1+SaATCC); (B) relative expression of QS gene in clinical isolate PaVBB in cross culture compared to paired culture (PaVBB+SaATCC compared to PaVBB+SaVBB). A relative expression change of more than twofold was considered significant.

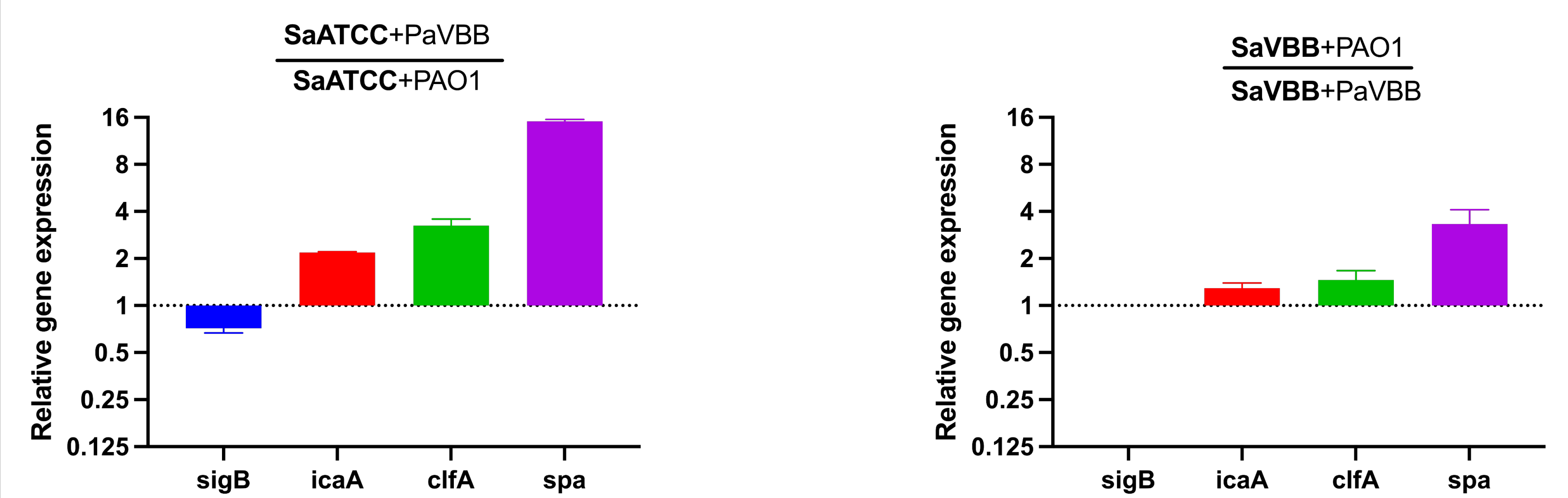


Fig. 3. *Staphylococcus aureus* gene expression when co-cultured with reference strain PAO1 or clinical isolate PaVBB at 72h. (A) relative expression of SaATCC genes in cross culture compared to reference biofilm (SaATCC+ PaVBB compared to SaATCC+ PAO1); (B) relative expression of genes in clinical isolate SaVBB in cross culture compared to paired culture (SaVBB+PAO1 compared to SaVBB+PaVBB). A relative expression change of more than twofold was considered significant.

Conclusion

- Clinical isolates seem adapted to each other, possibly because Pa QS genes and Sa spa gene (known to impair Pa biofilm formation) are less expressed in paired cultures than in cross cultures. This is also coherent with the lack of expression of the stress-response related gene sigB in the clinical Sa.

References

- Filkins *et al.* 2015 J Bacteriol 197(14):2252-64.
- Diaz Iglesias *et al.* 2019 Antimicrob Agents Chemother 63: e02204-19.

Acknowledgments

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