EUROBIOFILMS 2019

Glasgow, UK

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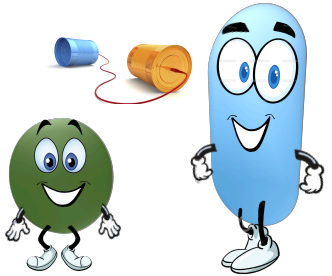
Universidade do Minho
Escola de Engenharia

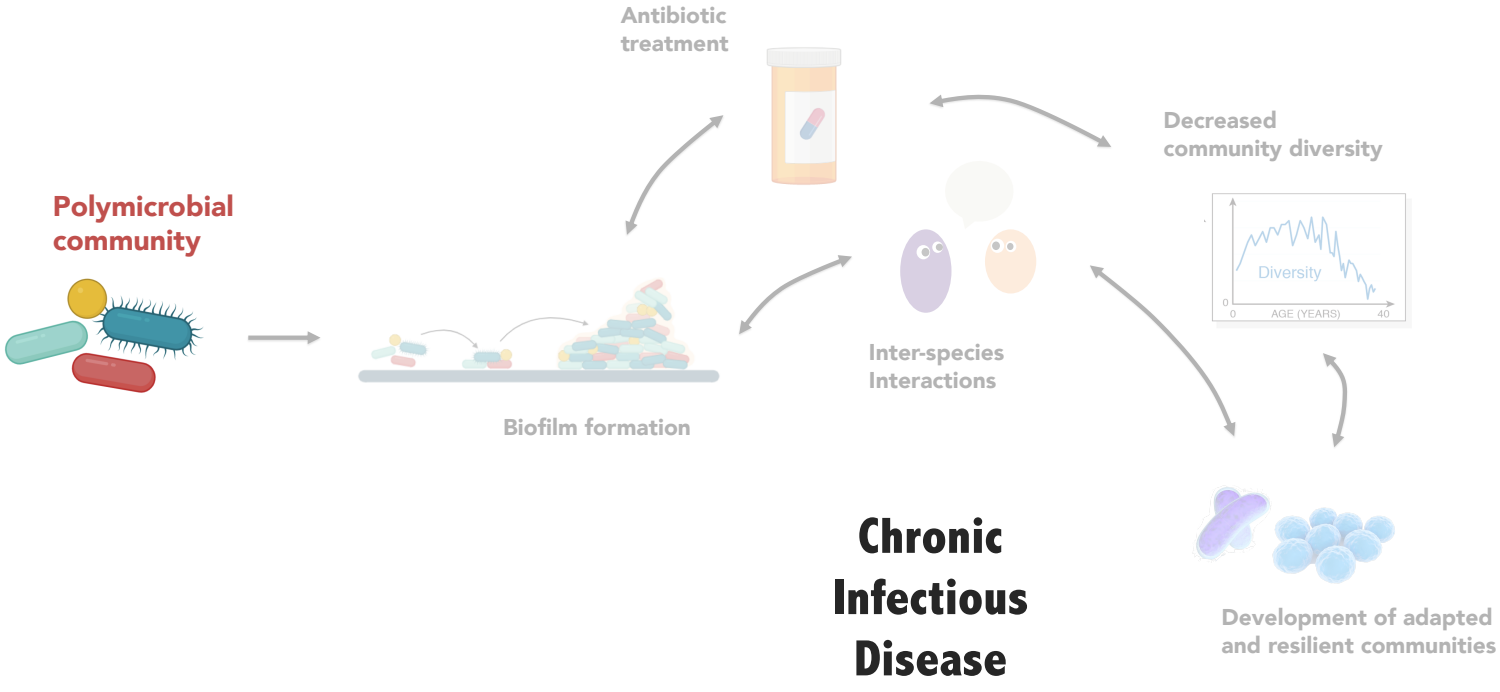
Viable but non-culturable state: a strategy for *Staphylococcus aureus* survivable in dual-species biofilms with *Pseudomonas aeruginosa* ?

Andreia Patrícia Magalhães, Tânia Grainha, Ana Margarida Sousa, Ângela França,

Nuno Cerca and Maria Olivia Pereira



Polymicrobial Biofilm Infections



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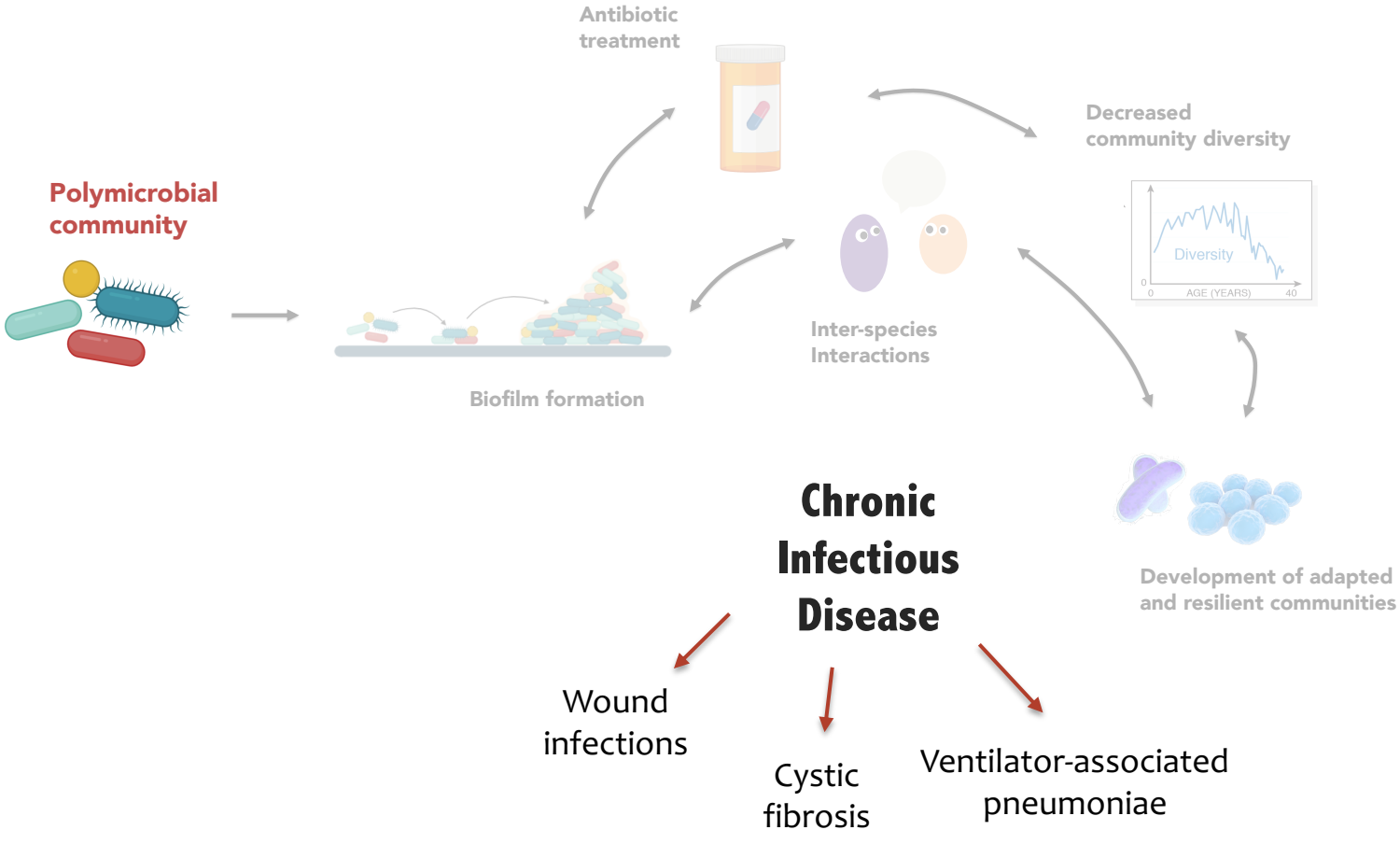

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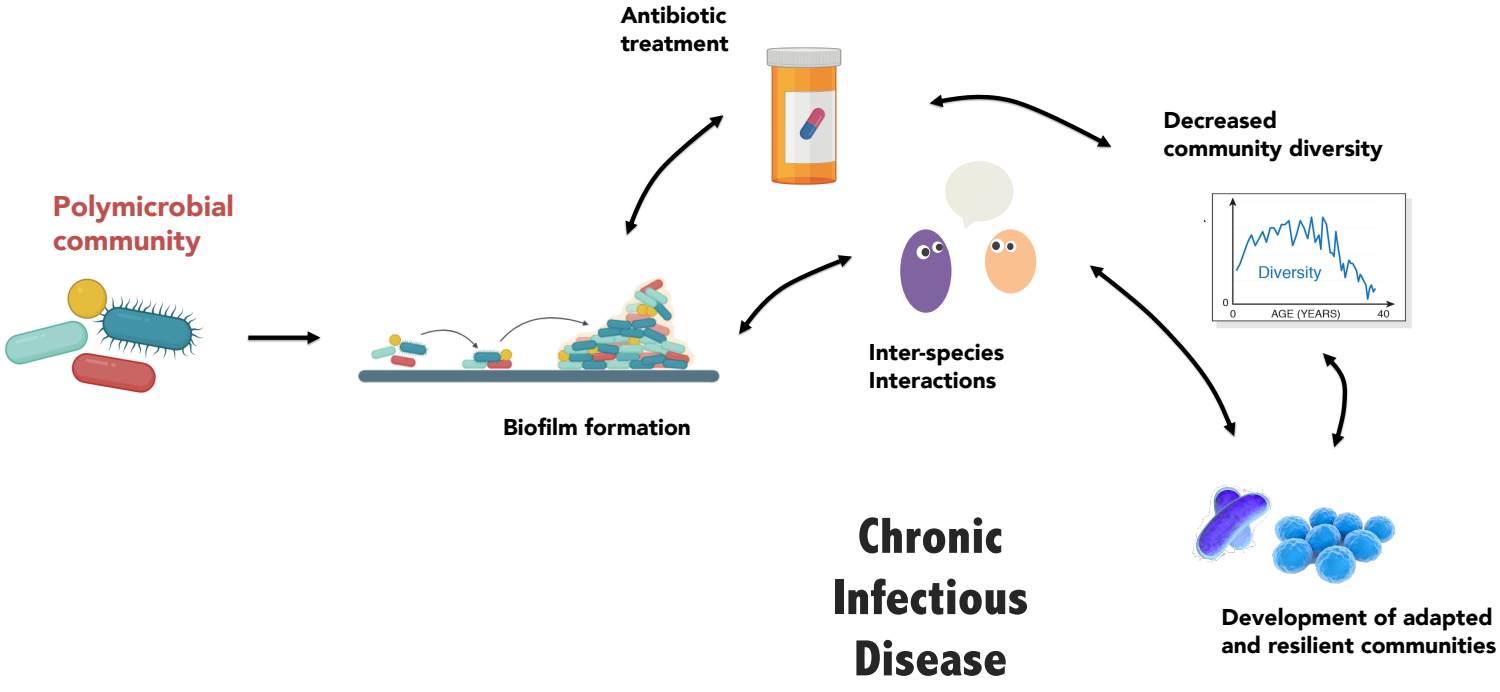

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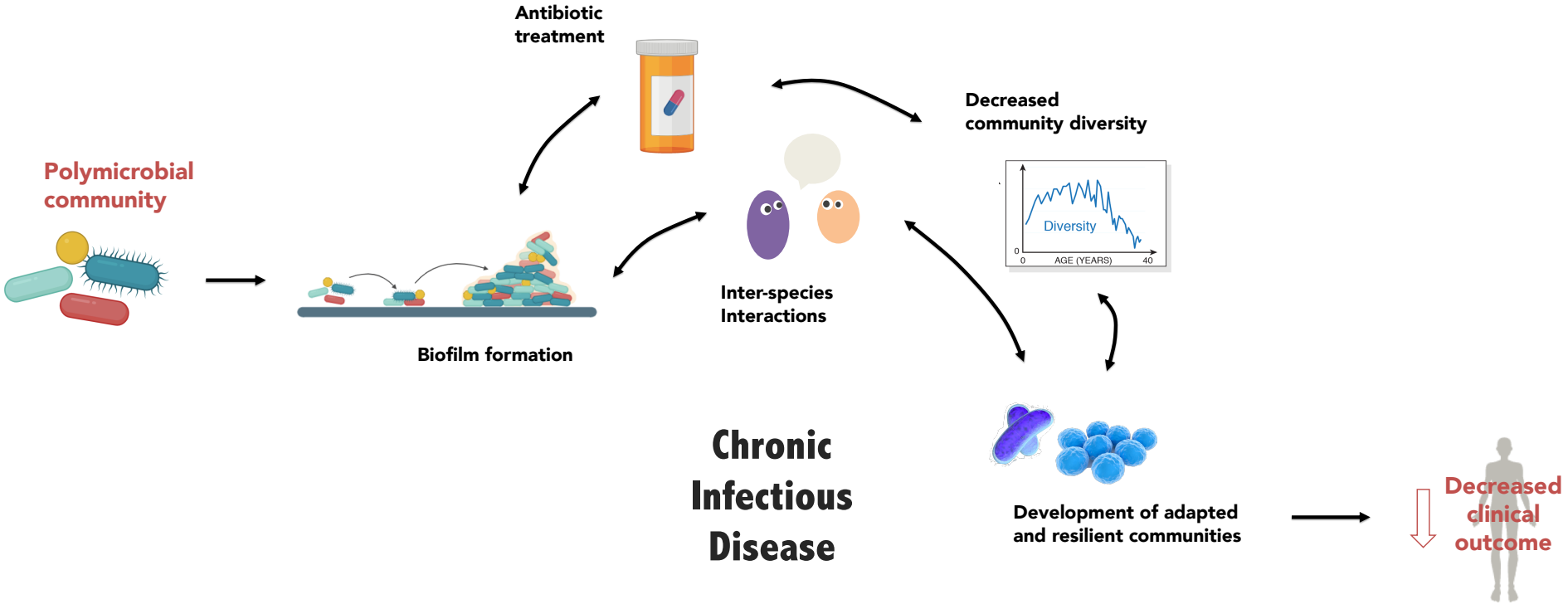

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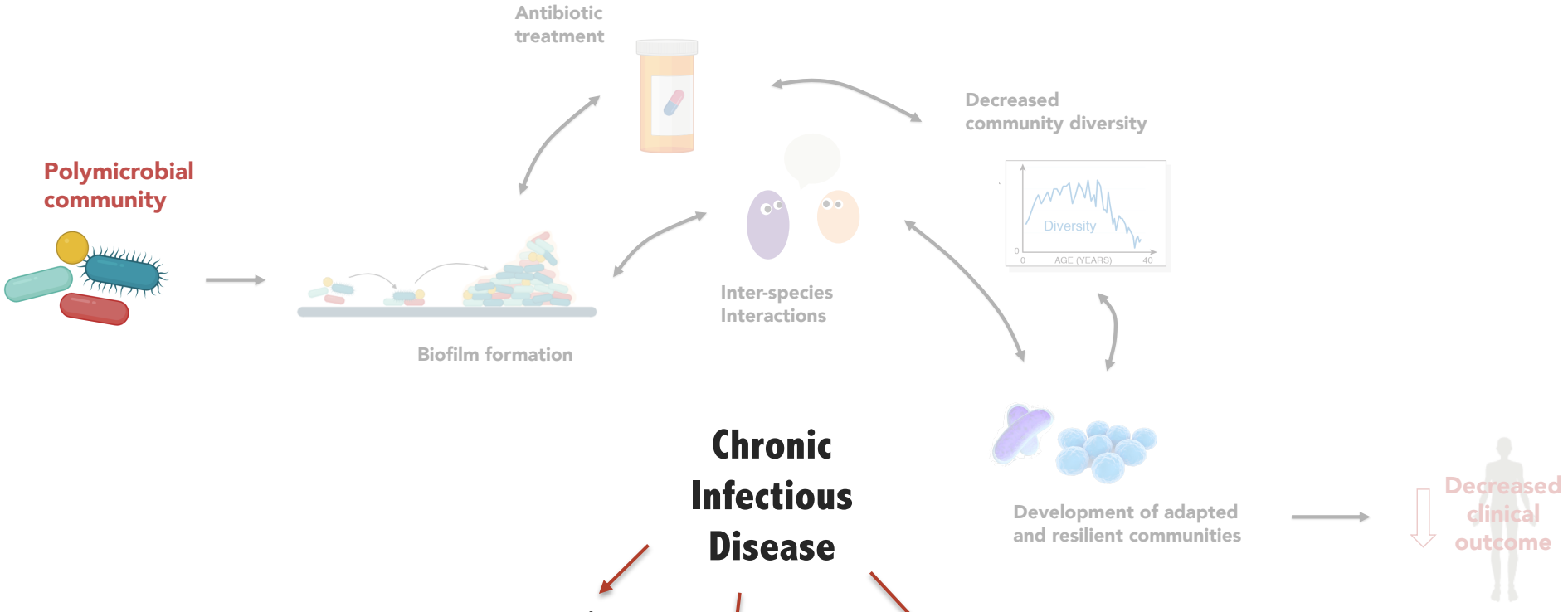
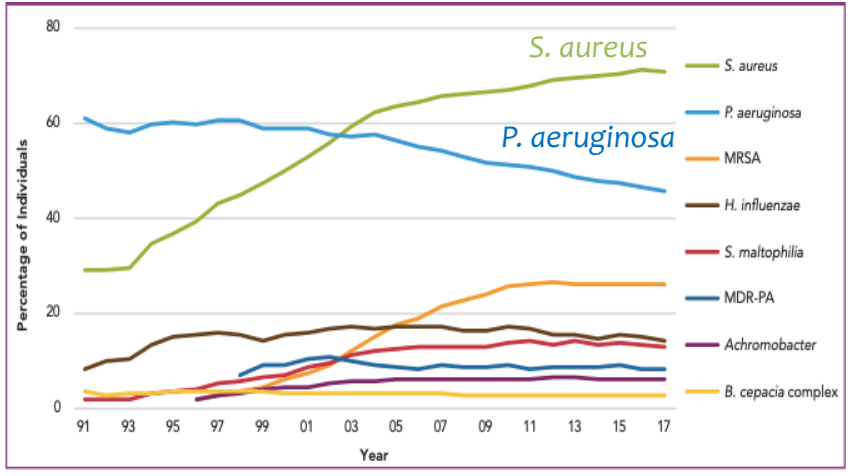


Polymicrobial Biofilm Infections

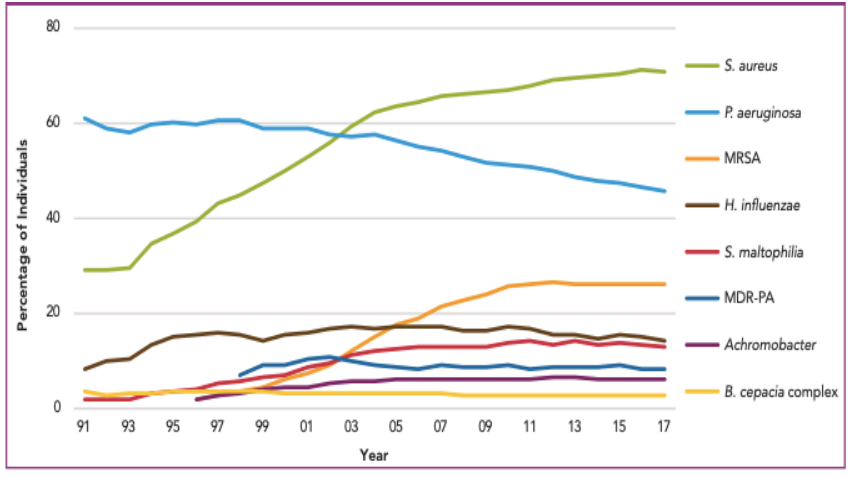
Cystic Fibrosis related-infections



Cystic Fibrosis Annual Data Report 2017

Polymicrobial Biofilm Infections

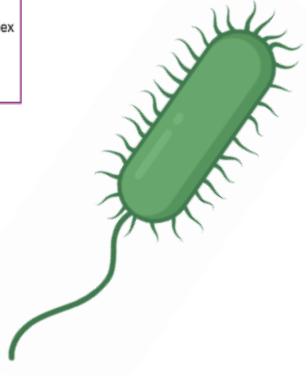
Cystic Fibrosis related-infections



Cystic Fibrosis Annual Data Report 2017

Staphylococcus aureus

- Prevalent among people with and without CF
- ~ 20 % of strains are multidrug-resistant



Pseudomonas aeruginosa

- A leading cause of airway infection
- Associated with a decline in lung function
- ~18 % of strains are multidrug-resistant

AIM:

To investigate the community dynamics of *Pseudomonas aeruginosa* and *Staphylococcus aureus*, two common co-infecting pathogens in cystic fibrosis infections, growing as dual-species biofilms.

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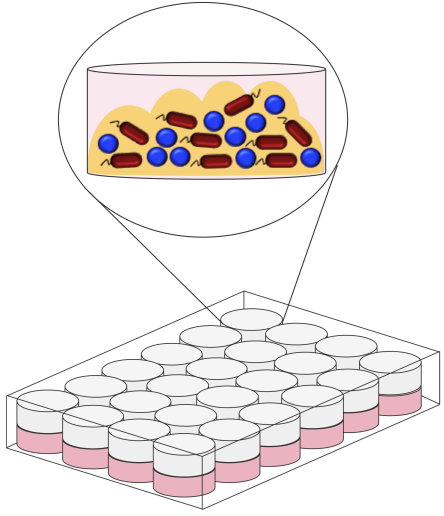
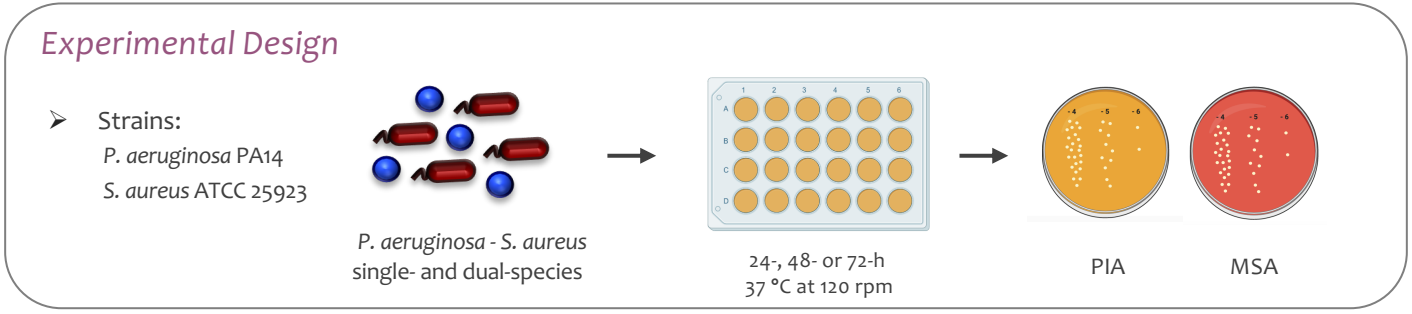
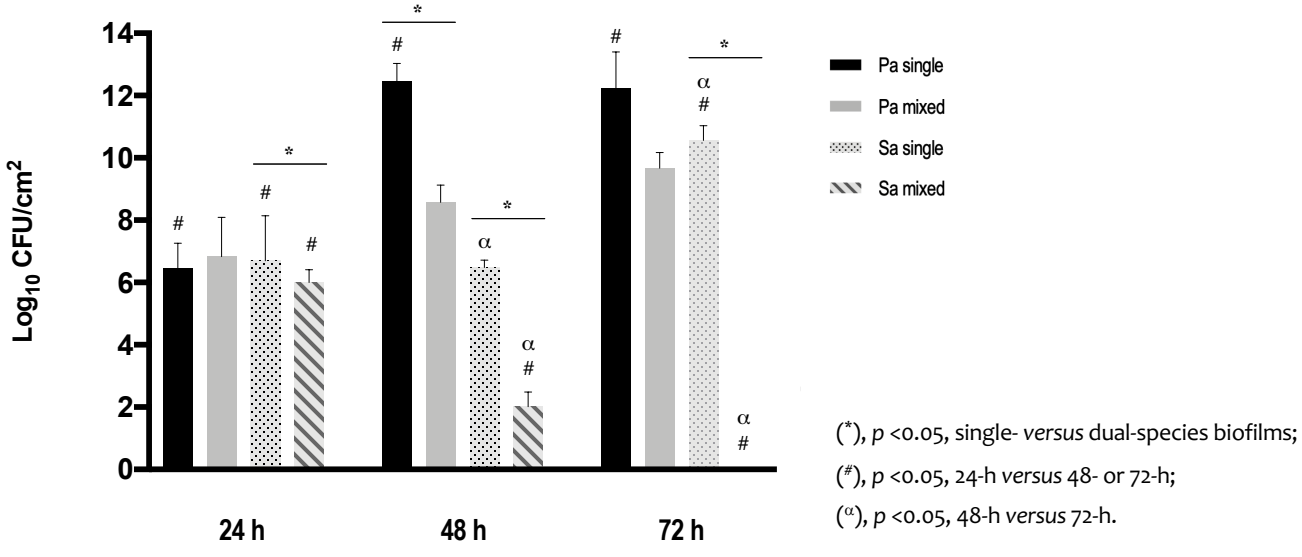


- Biofilm structure
- Microbial composition
- Gene expression profile

Biofilm quantification by plate counts: single- and dual-species





(*), $p < 0.05$, single- *versus* dual-species biofilms;

(#), $p < 0.05$, 24-h *versus* 48- or 72-h;

(α), $p < 0.05$, 48-h *versus* 72-h.

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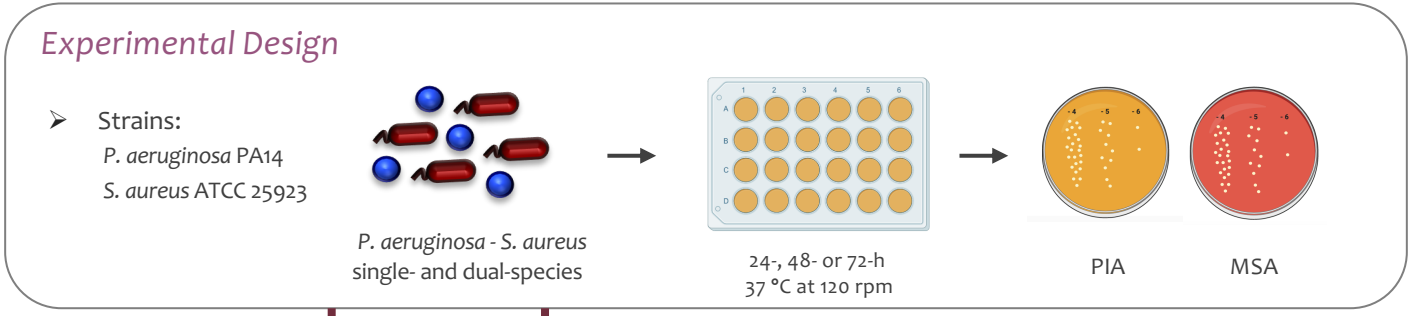

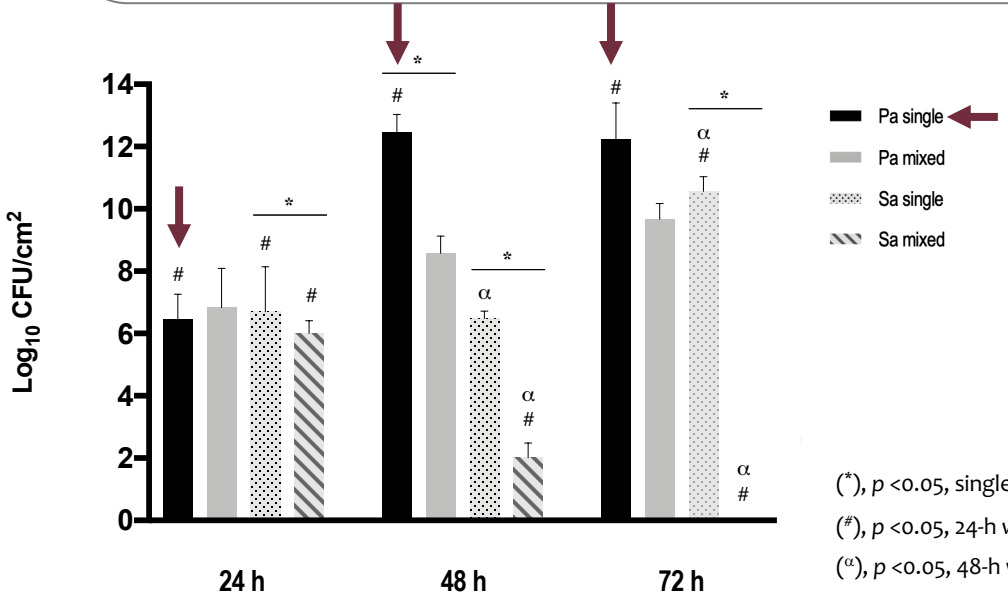

- Single-species biofilm increased their number from 24- up to 72-h of growth

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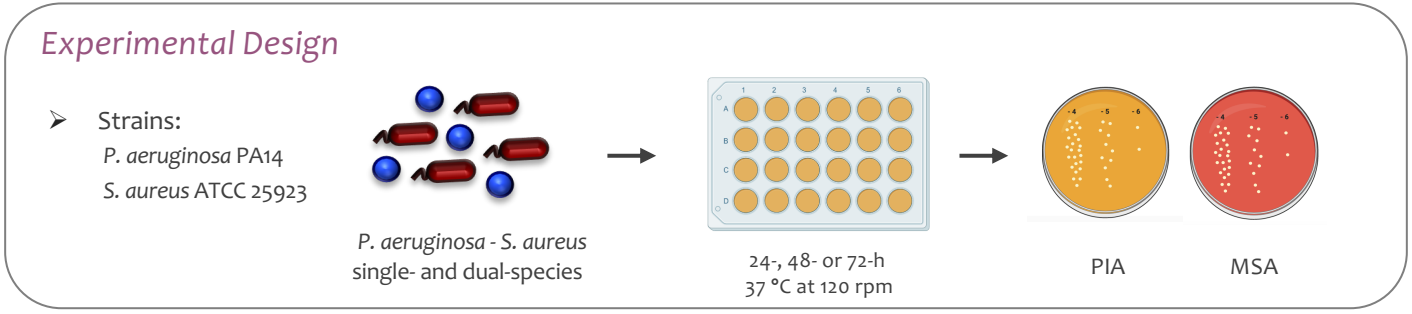

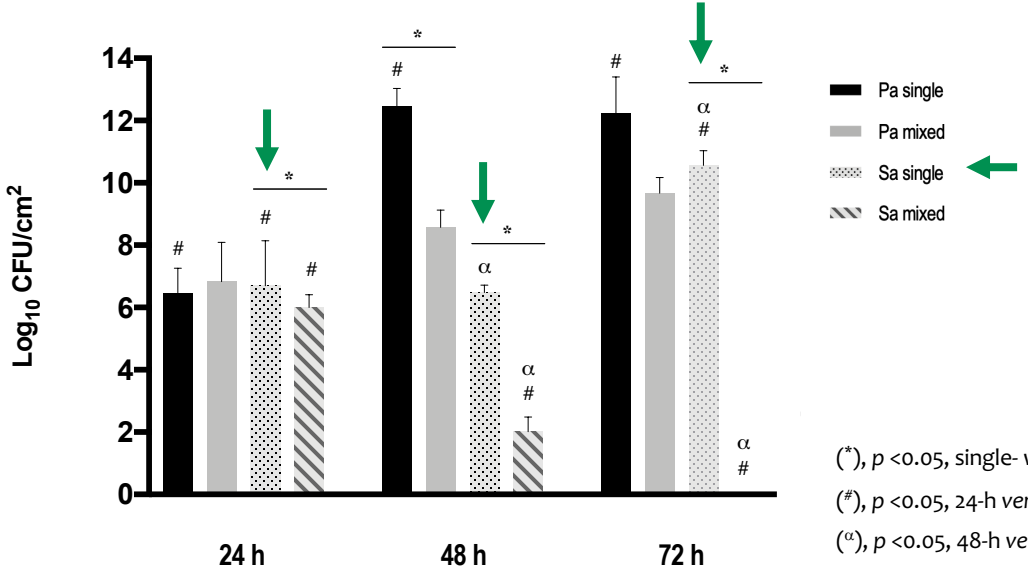

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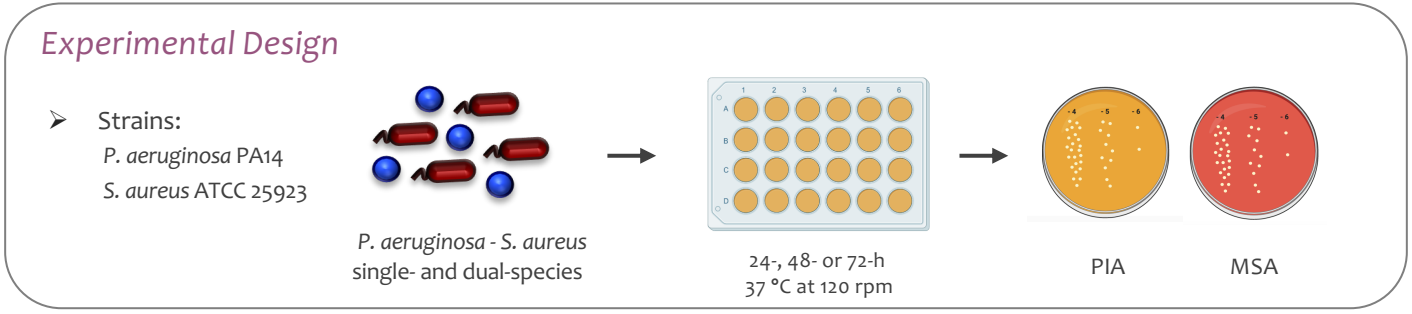

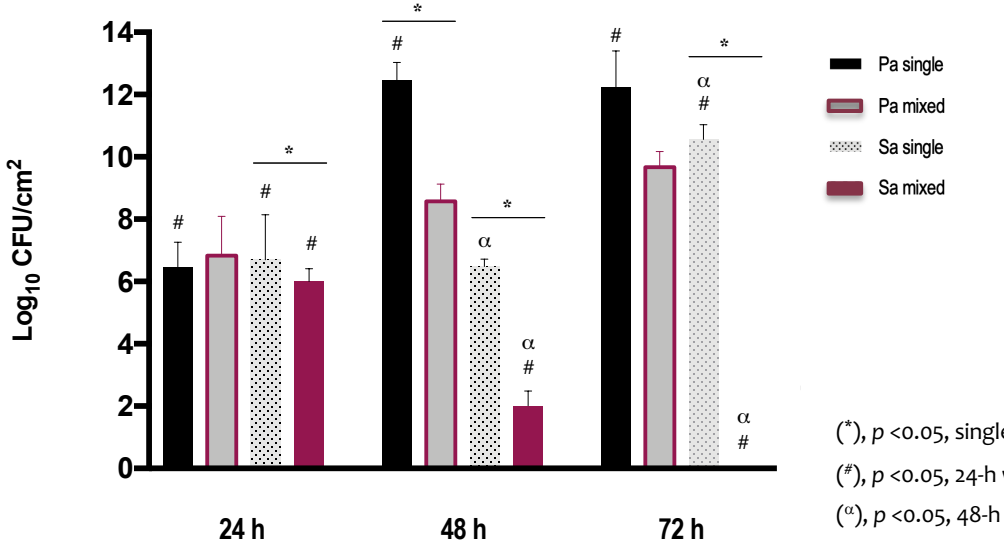

- *S. aureus* growth significantly decreased from 24- to 48- and 72-h in the presence of *P. aeruginosa*
- *P. aeruginosa* growth was not affected by the presence of S. aureus

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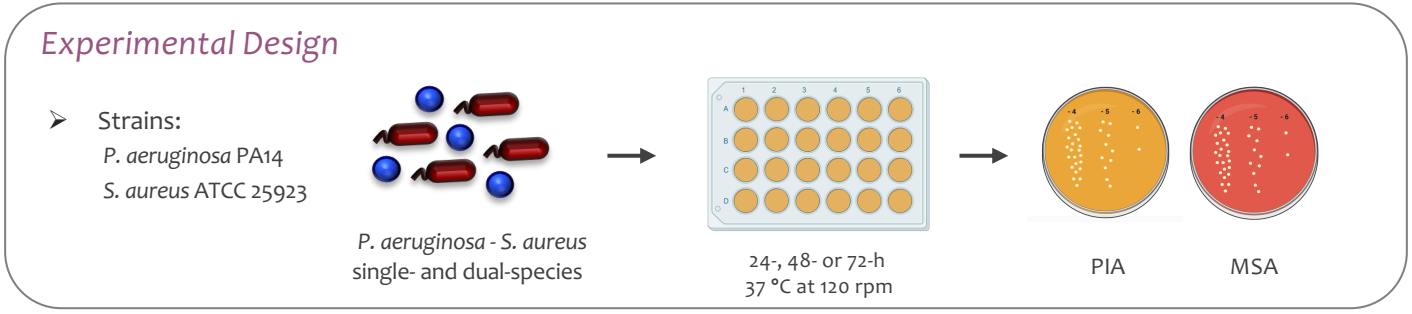
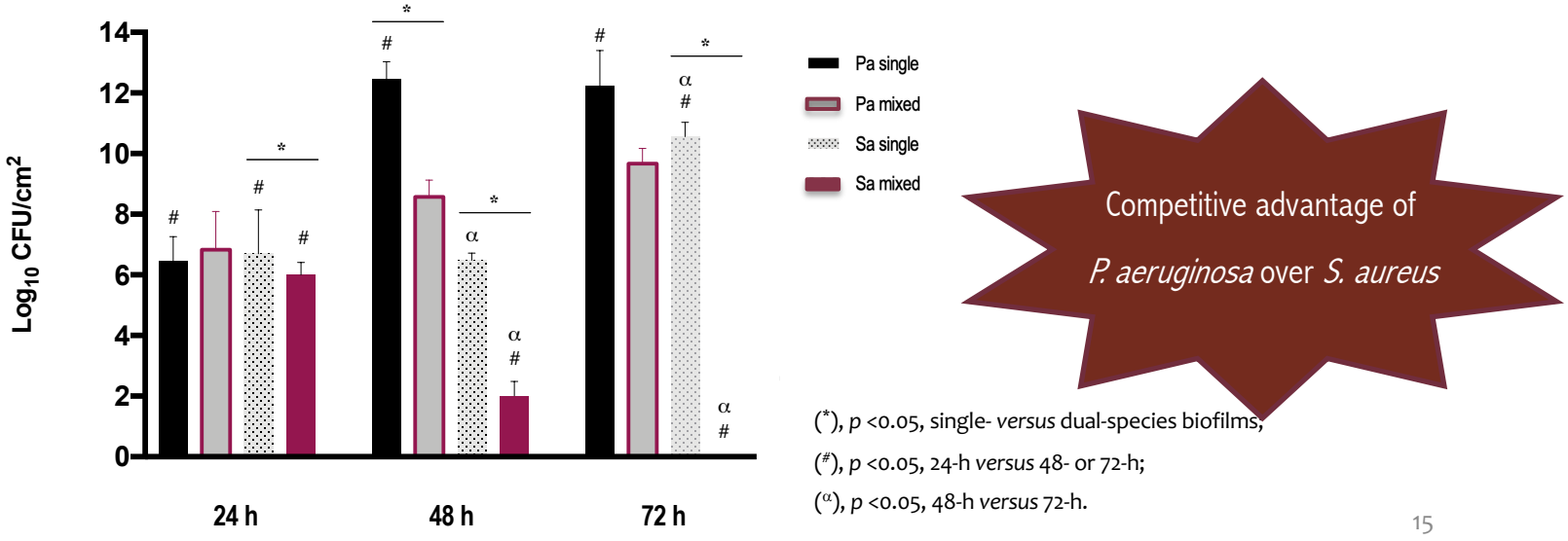
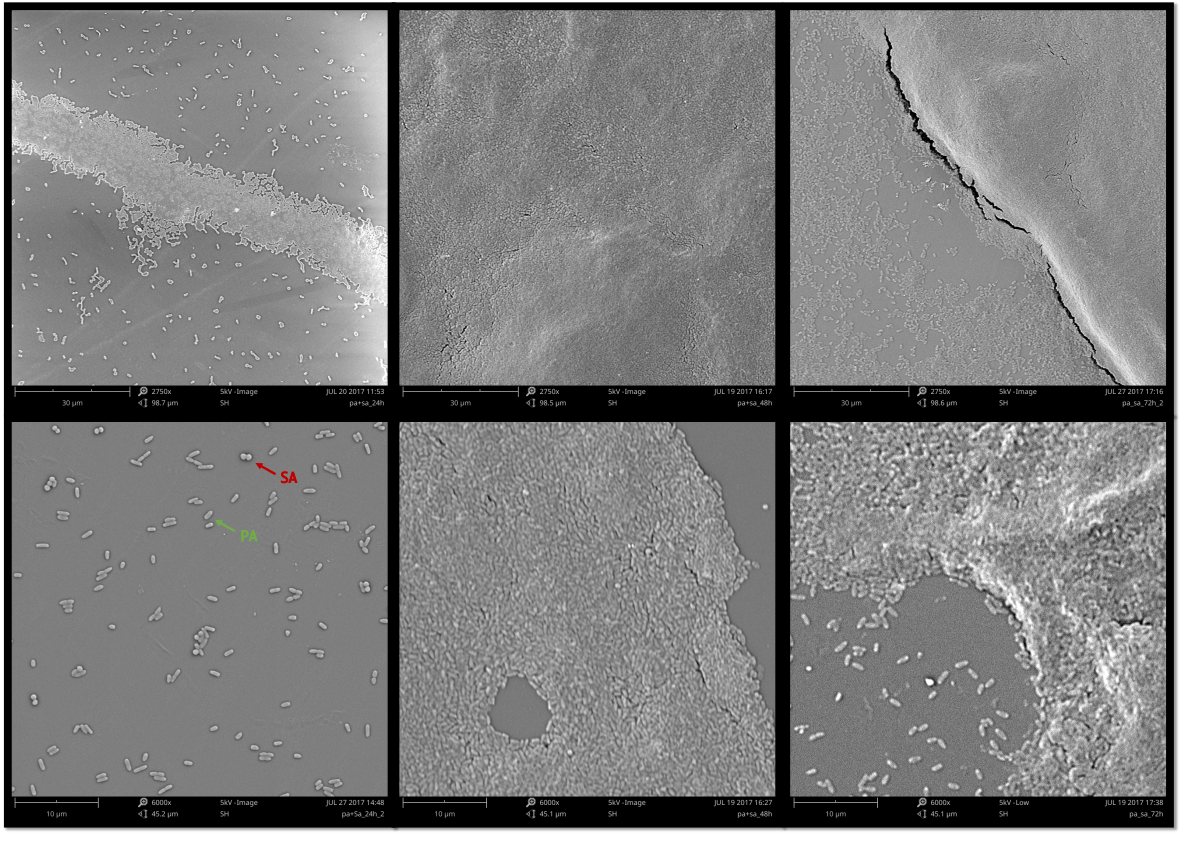




Biofilm structure: SEM analysis

Magnification 2750 x

Magnification 6000 x



24-h-old **48-h-old** **72-h-old**

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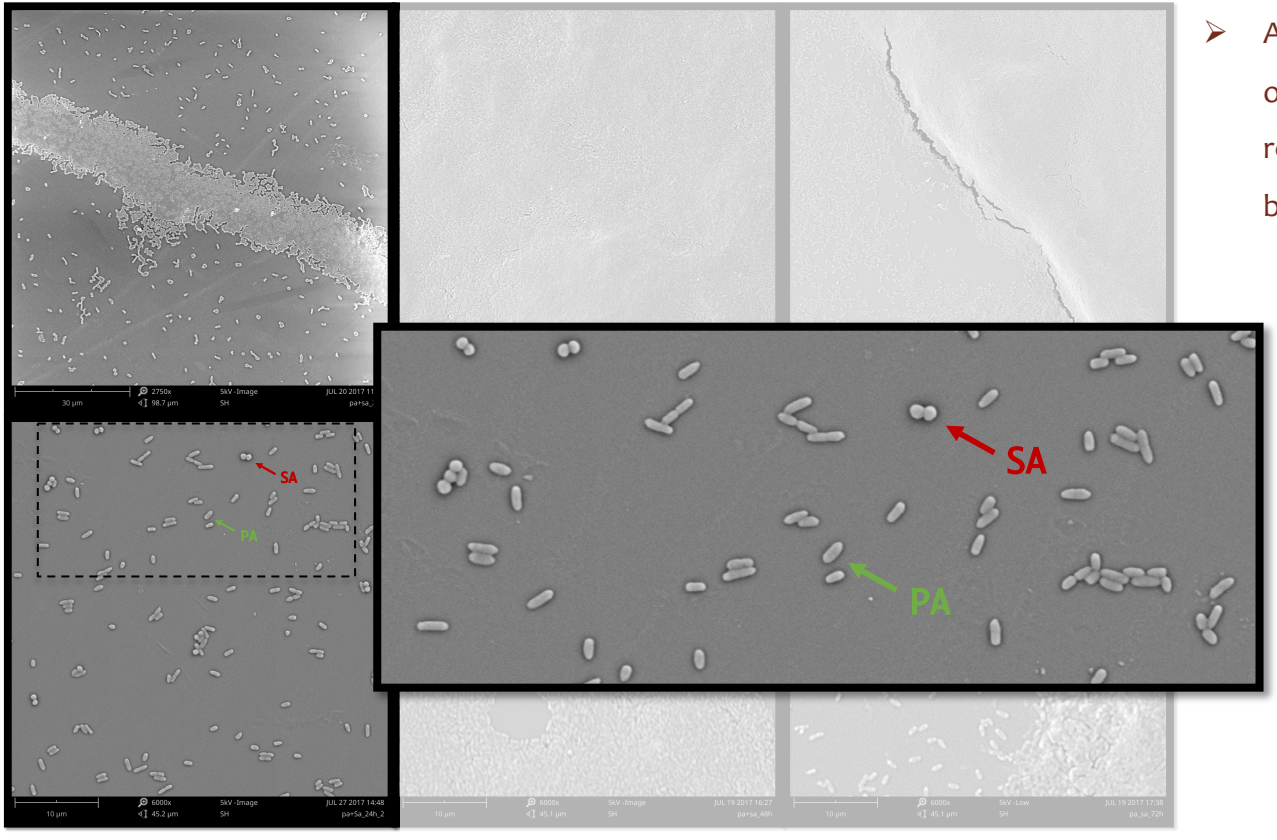

24-h-old

48-h-old

72-h-old

- A non-contiguous layer of cells is observed, representing the initial biofilm stages.
- Both species were detected in the dual-species consortia

Biofilm structure: SEM analysis

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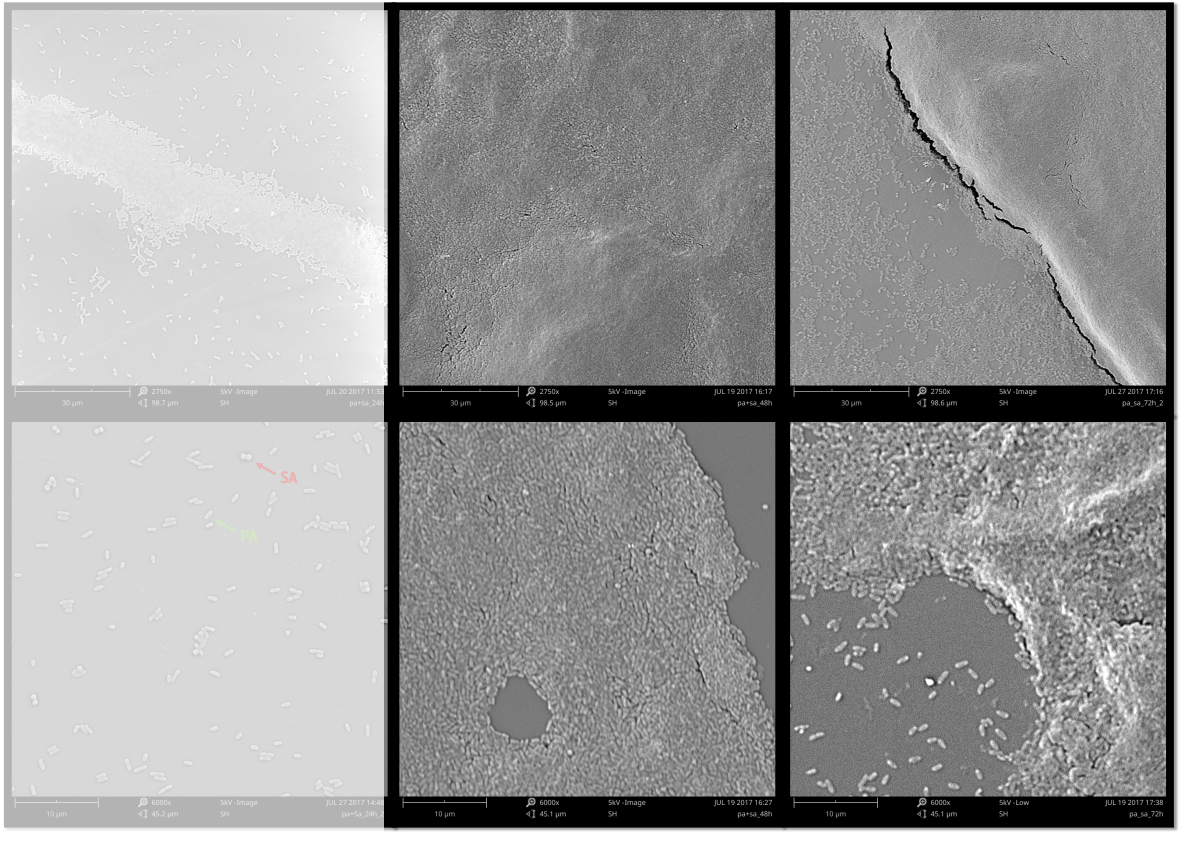
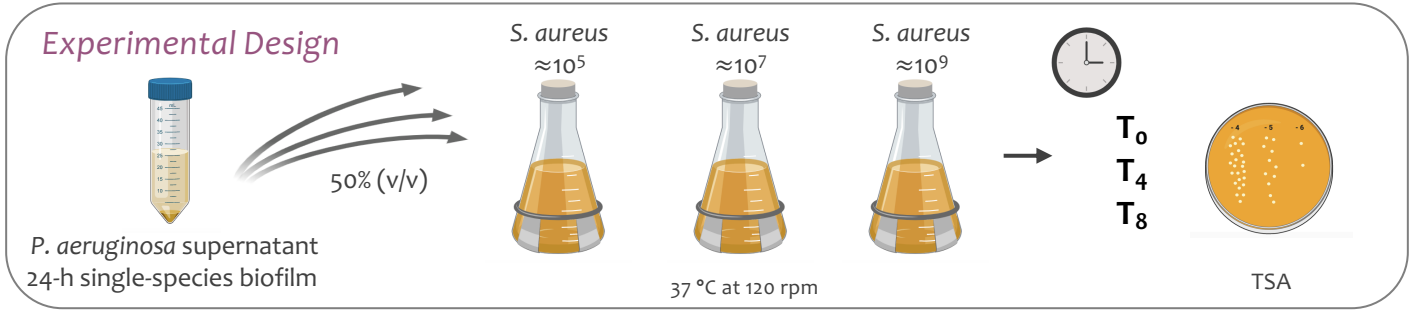
Magnification 6000 x

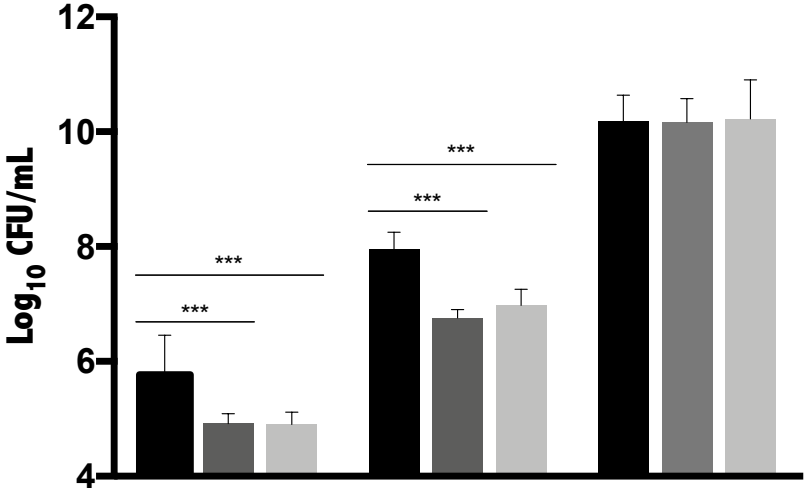


24-h-old | 48-h-old | 72-h-old

- Mature biofilms producing a thickness of co-aggregated cells surrounded by extracellular biofilm matrix

Effect of secreted compounds produced by *P. aeruginosa*

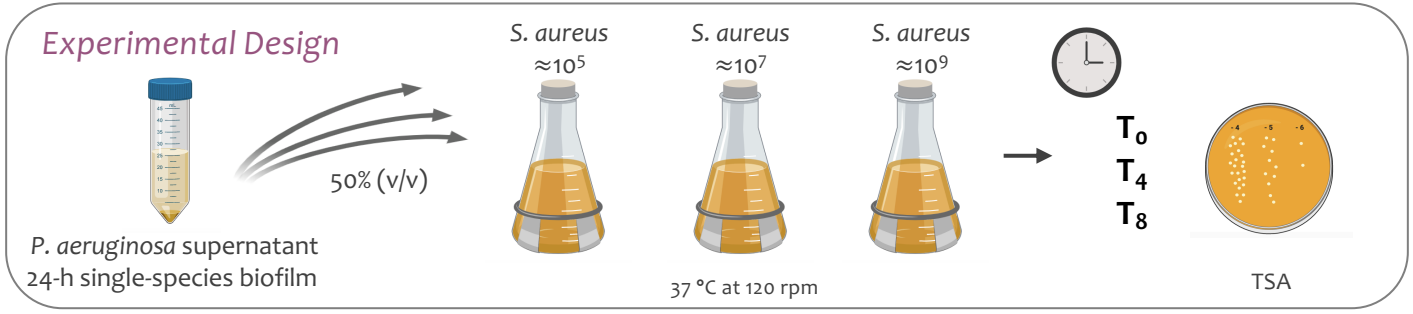


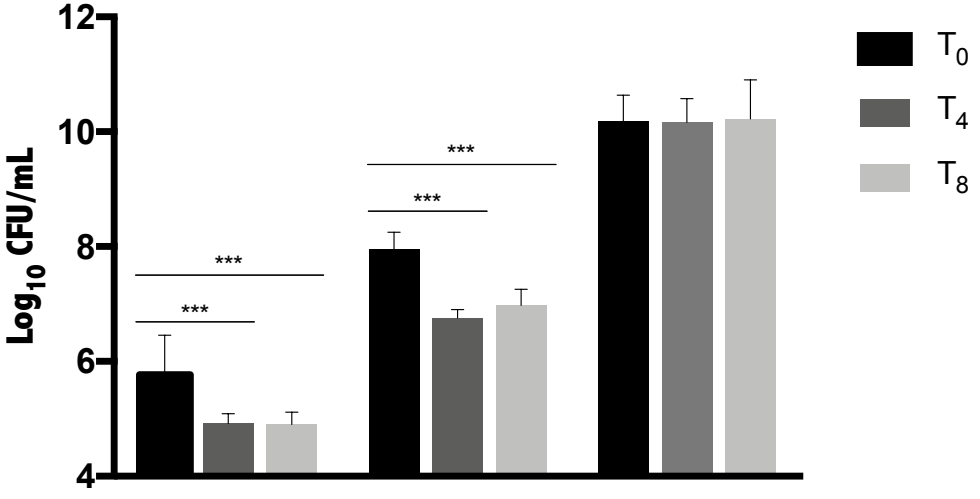


- Only 1 log of cell reduction (for 10^5 and 10^7 CFU/mL) was observed

(****) $p < 0.0001$, compared to T_0

Effect of secreted compounds produced by *P. aeruginosa*

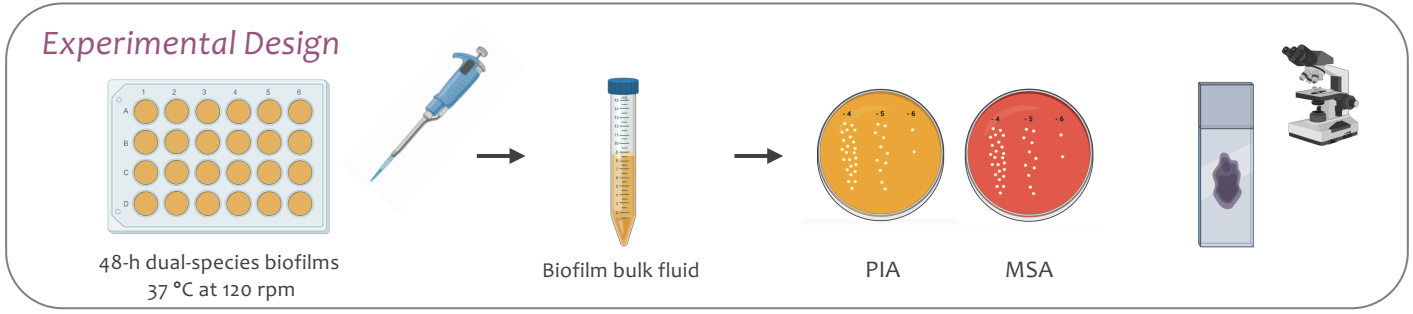


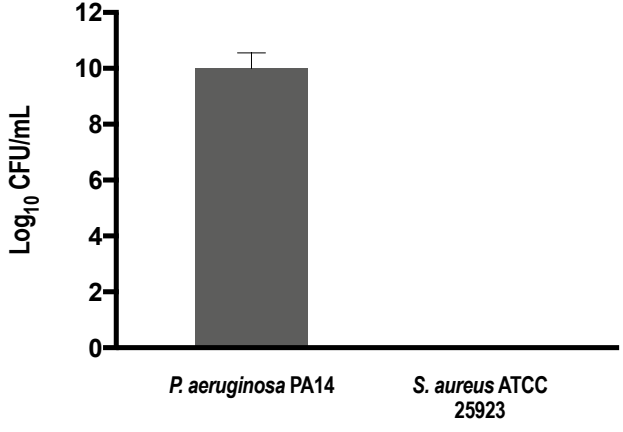


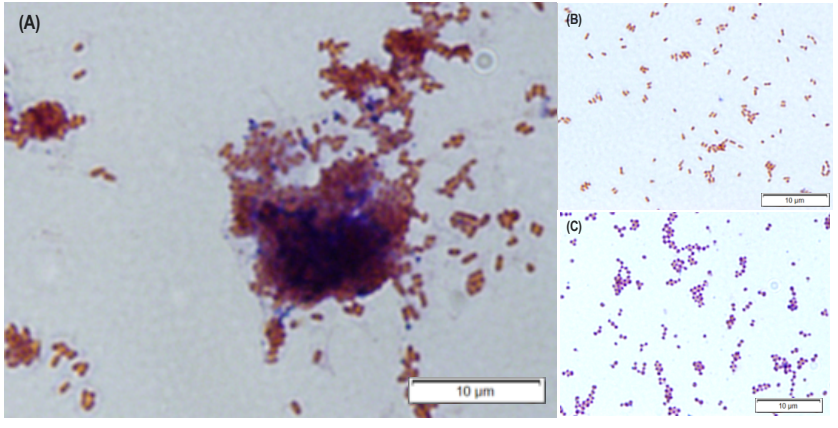
P. aeruginosa biofilm supernatant did not affected *S. aureus* growth

(****) $p < 0.0001$, compared to T_0

Biofilm bulk fluid quantification



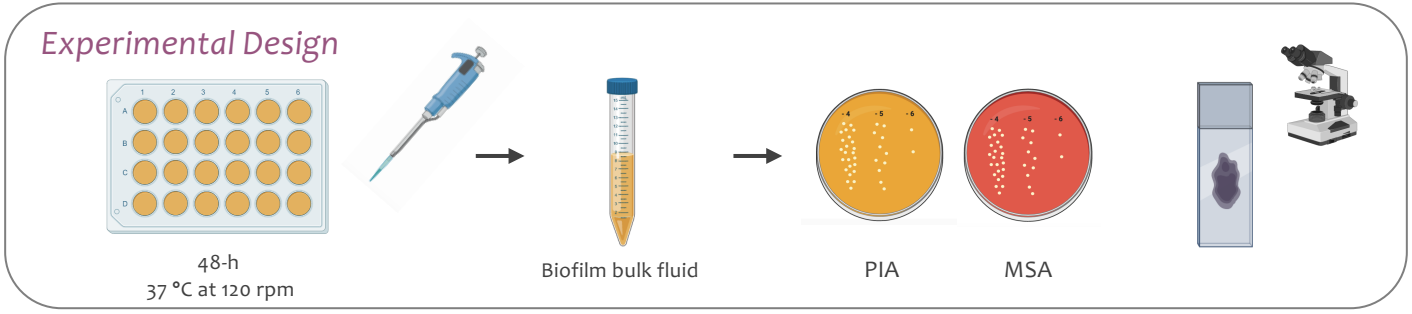


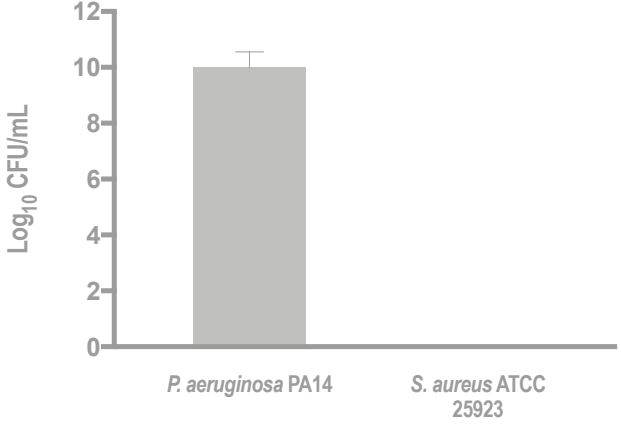


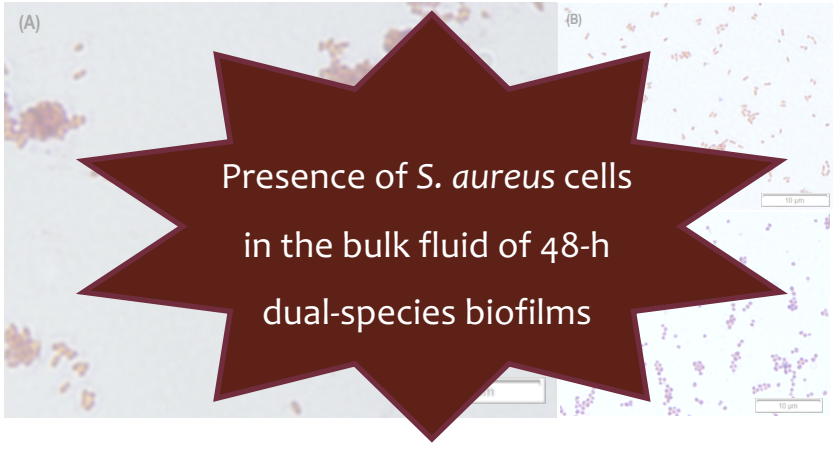
Dual- species biofilms

Single-species biofilm

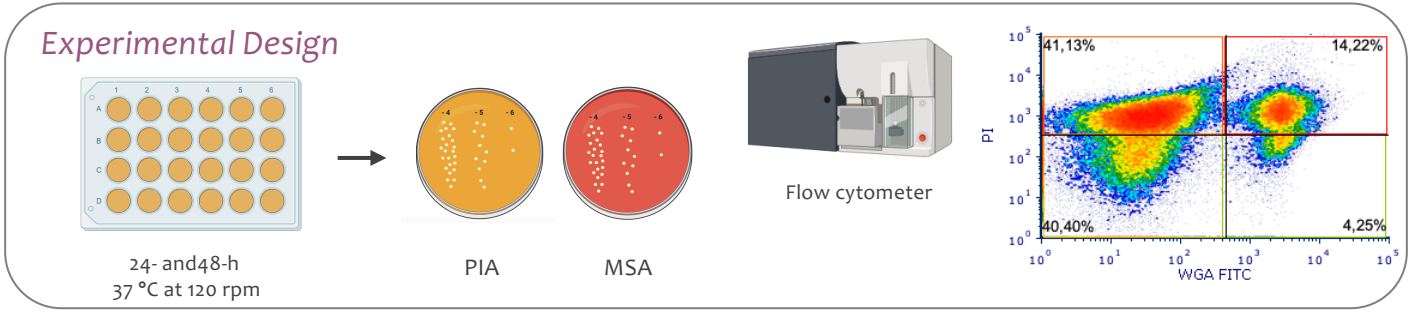
Biofilm bulk fluid quantification

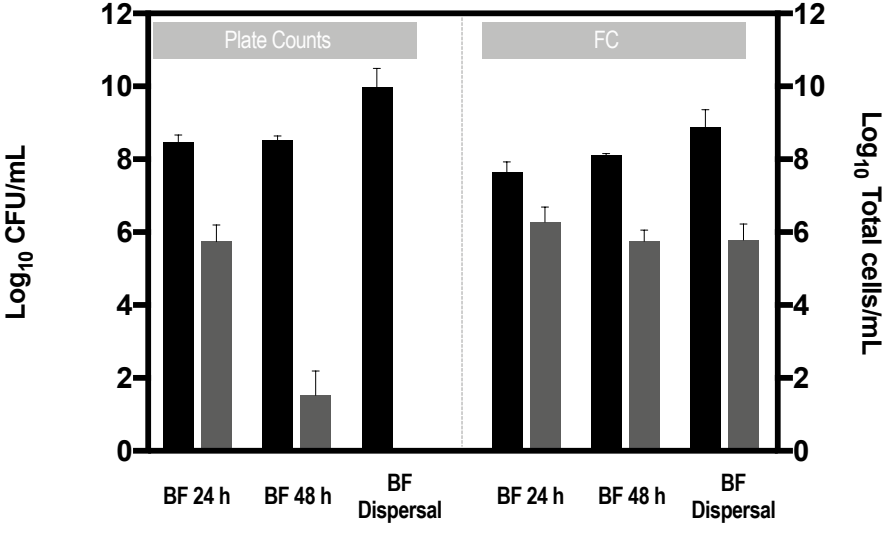






Quantitative assessment of individual populations within dual-species biofilms





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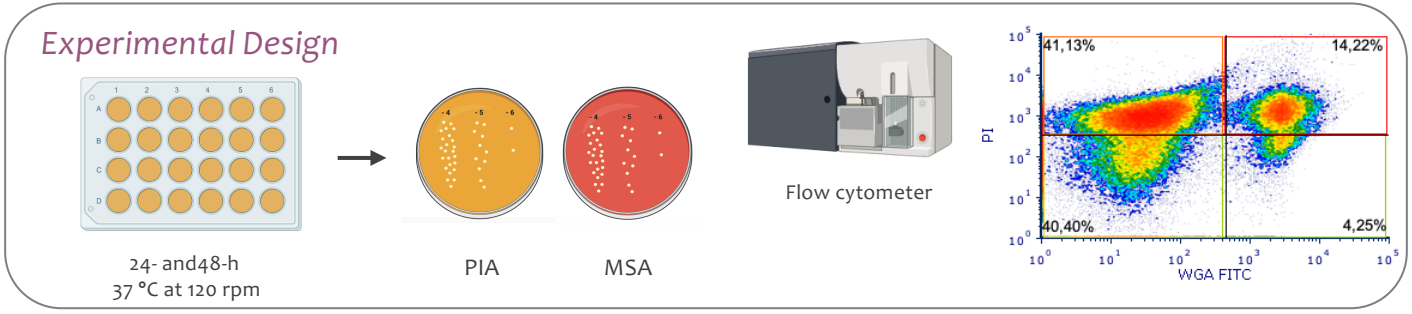

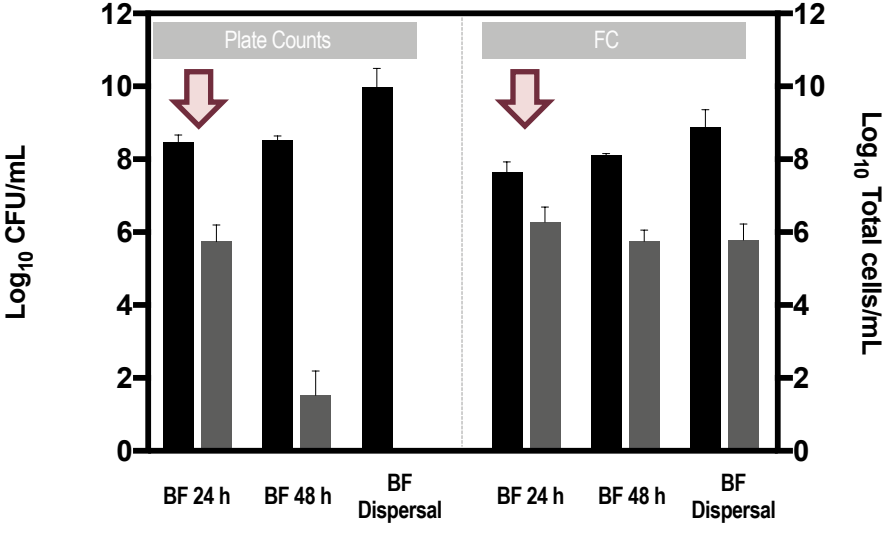

- For 24-h biofilms similar counts were detected by both methods

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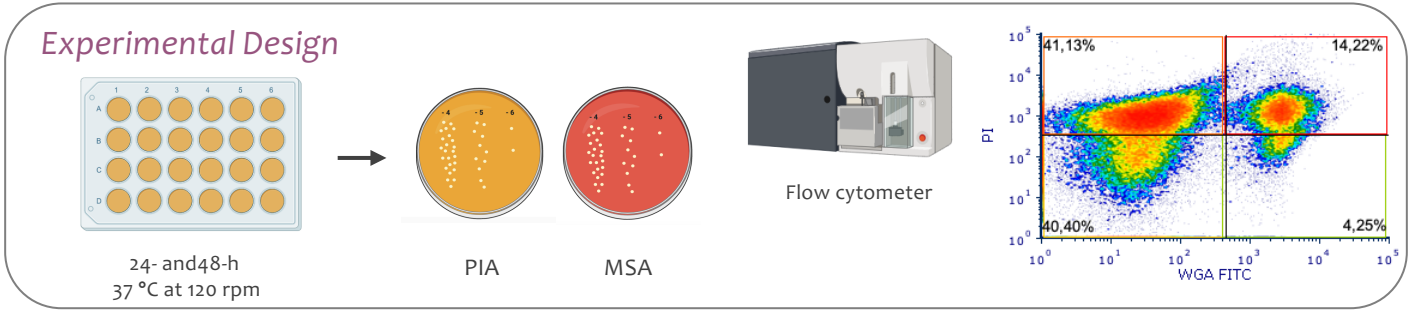

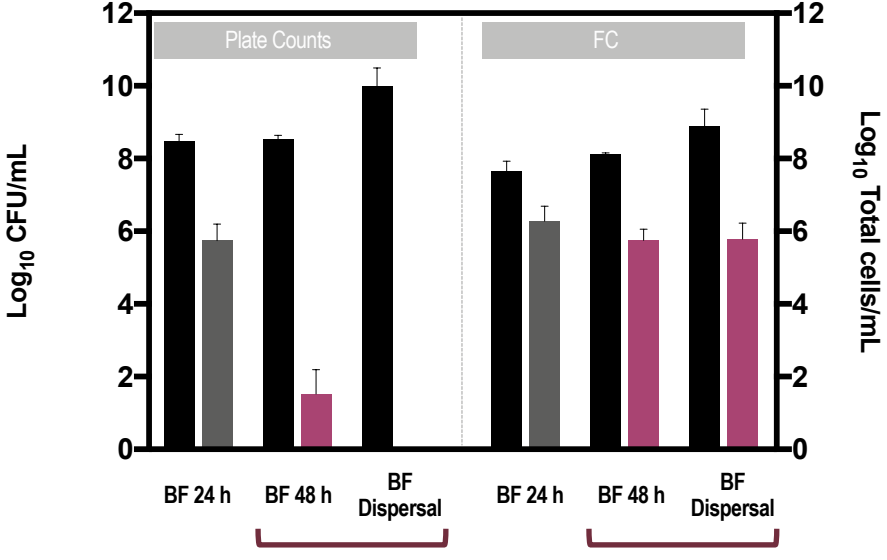

S. aureus cells were detected in high abundance by flow cytometry;

Presence of *S. aureus* VBNC

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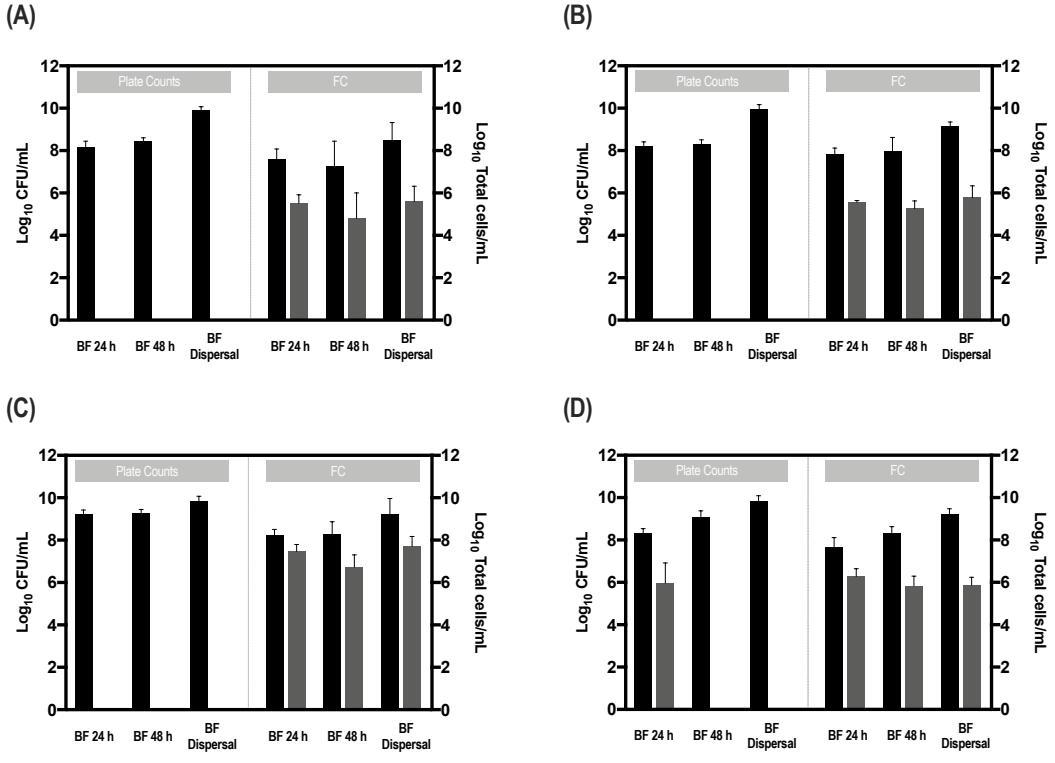

(A) *P. aeruginosa* 362668 mucoid and *S. aureus* ATCC 25923

(B) *P. aeruginosa* 362668 mucoid and *S. aureus* 352845

(C) *P. aeruginosa* 362668 non-mucoid and *S. aureus* ATCC 25923

(D) *P. aeruginosa* PA14 and *S. aureus* 352845.

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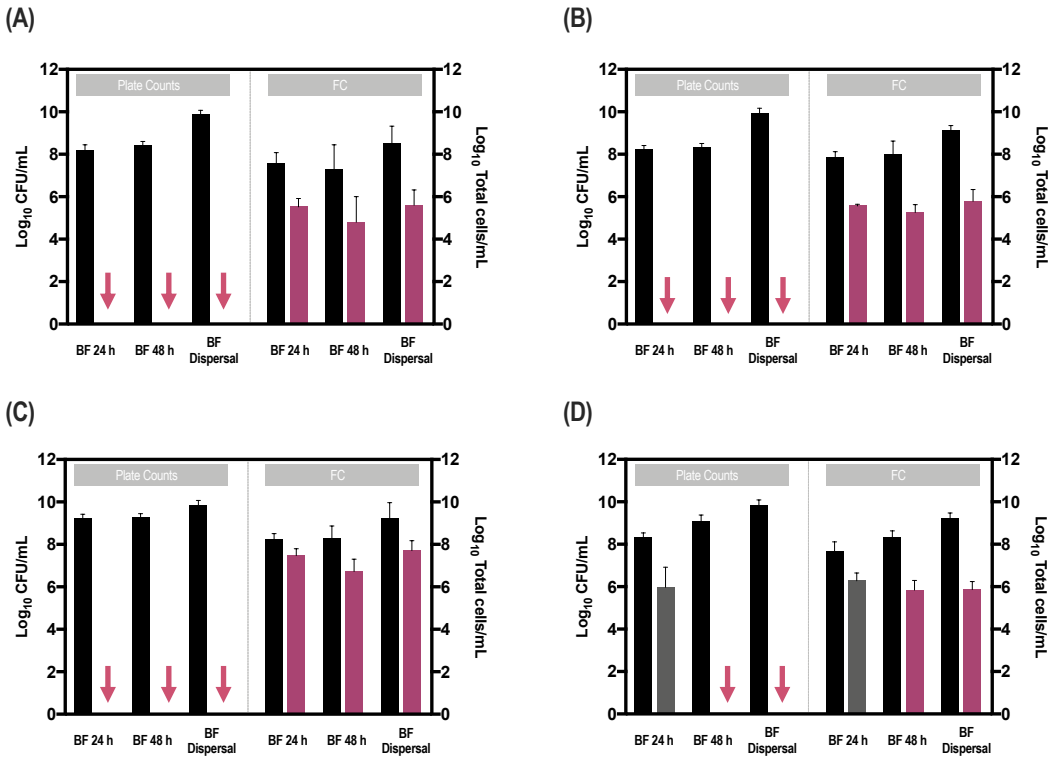

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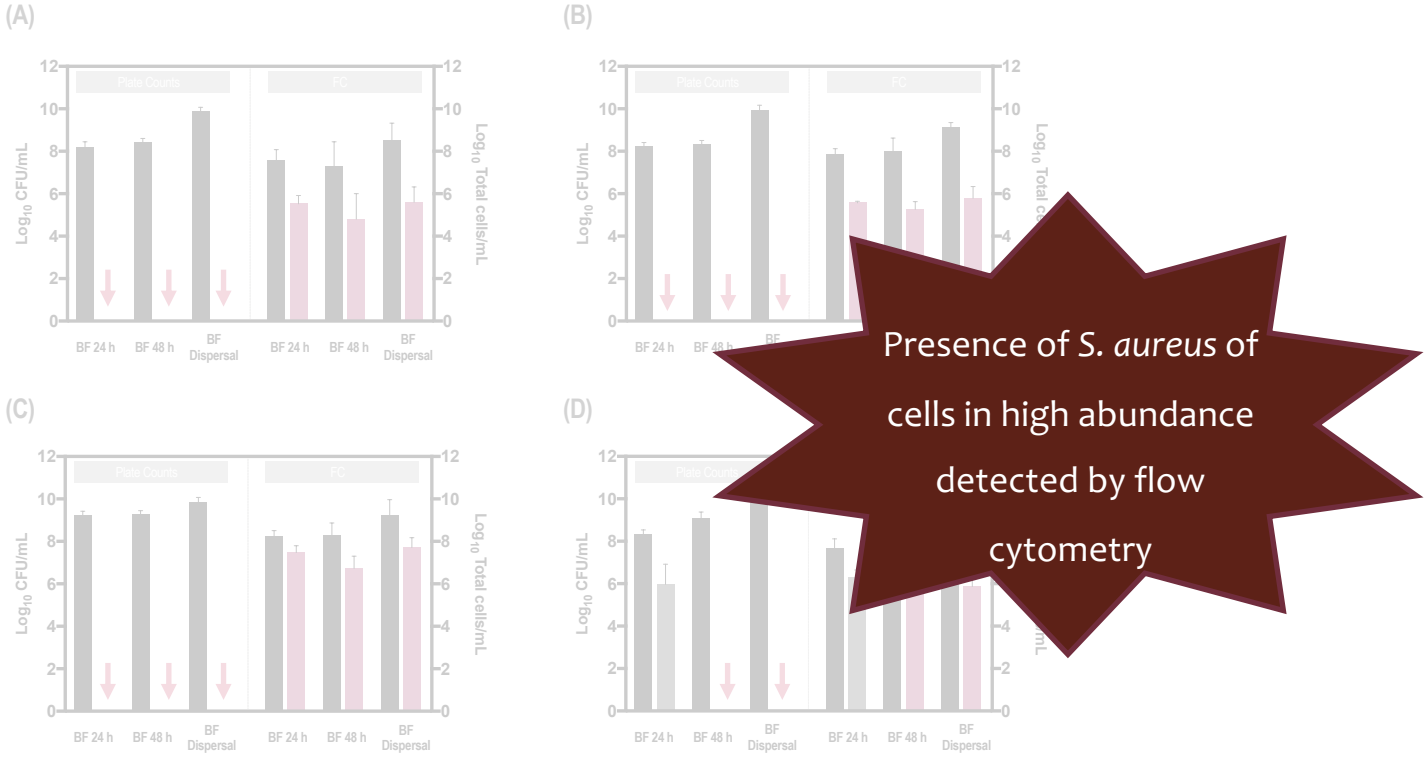

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Virulence expression in dual-species biofilms

P. aeruginosa virulence-related genes:

Gene	***Function***
pqsE	HQNO
rhlR	Virulence Regulator (Quorum sensing)
pvdE	Pyoverdine
toxA	Exotoxin A
lasI	Virulence Regulator (Quorum sensing)
algD	Alginate

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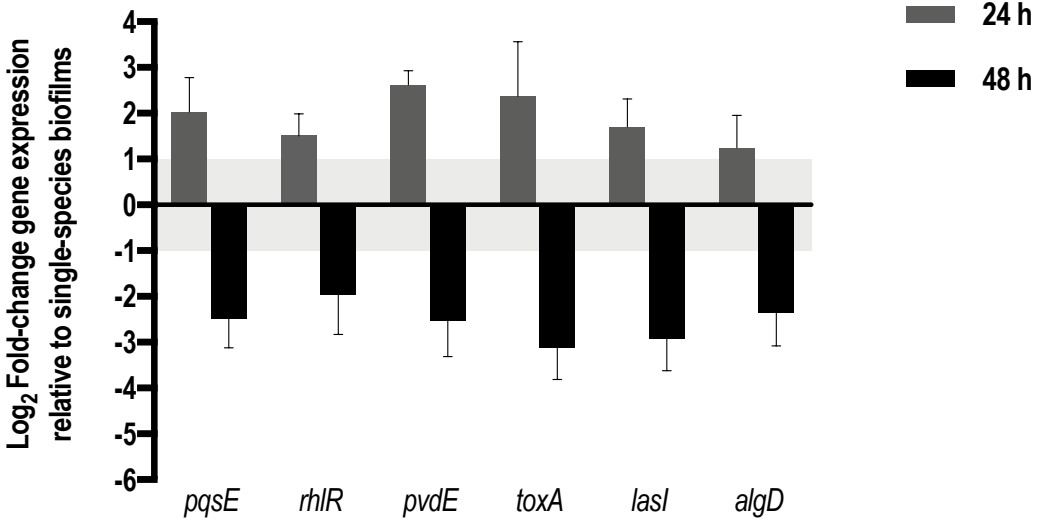

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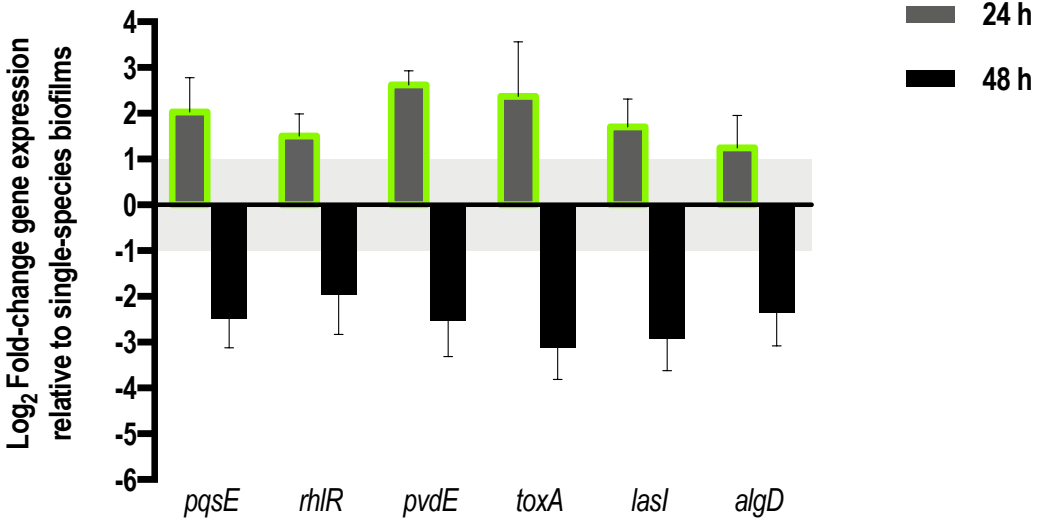

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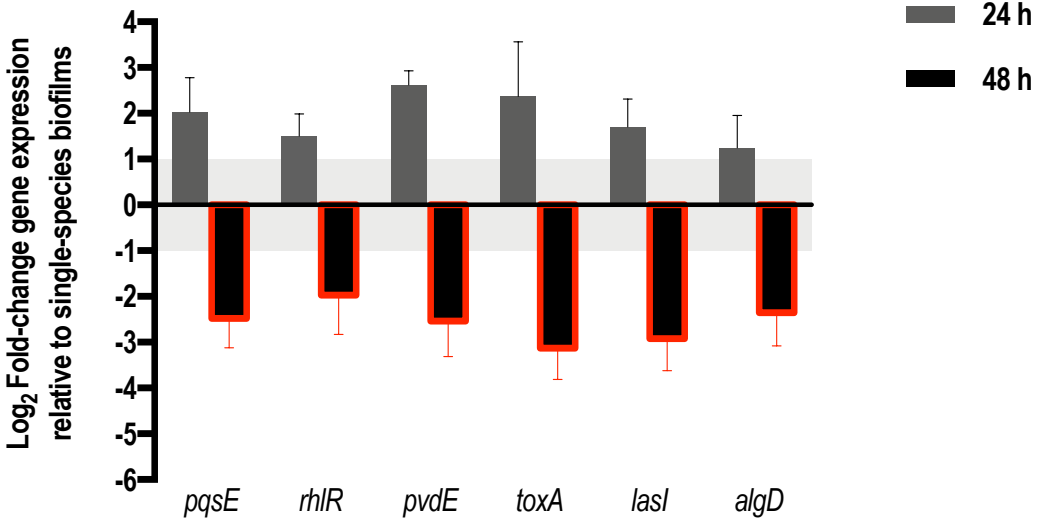

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Gene	***Function***
sodA	Stress Response
sarA	Virulence Regulator (Quorum sensing)
agrB	Virulence Regulator (Quorum sensing)
hld	Virulence Regulator (Quorum sensing)
icaA	Biofilm formation (PNAG production)
hla	Alfa-hemolysin
uspA	Stress Response

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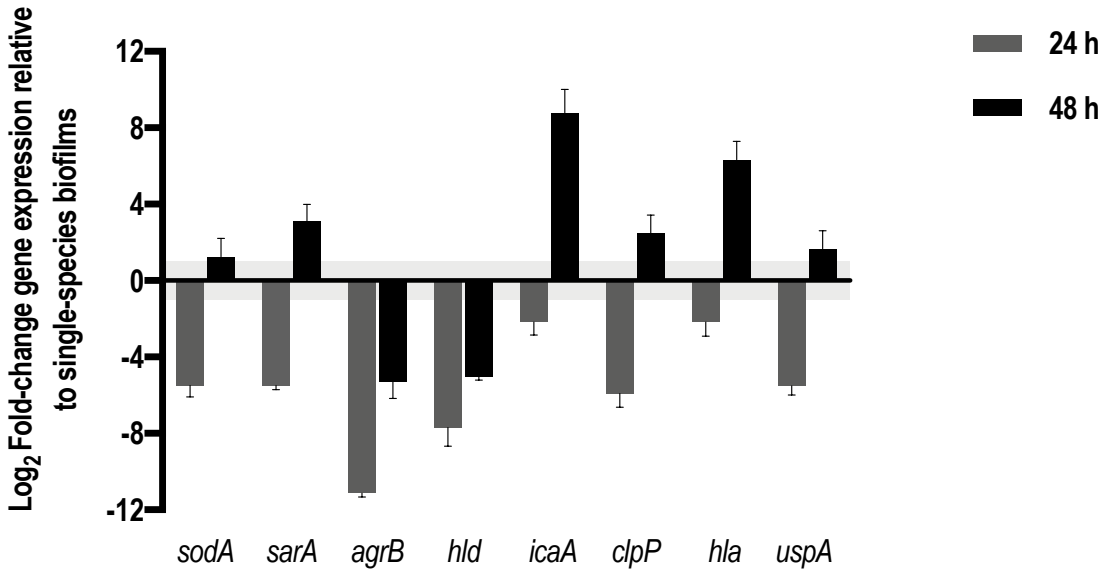

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Virulence expression in dual-species biofilms

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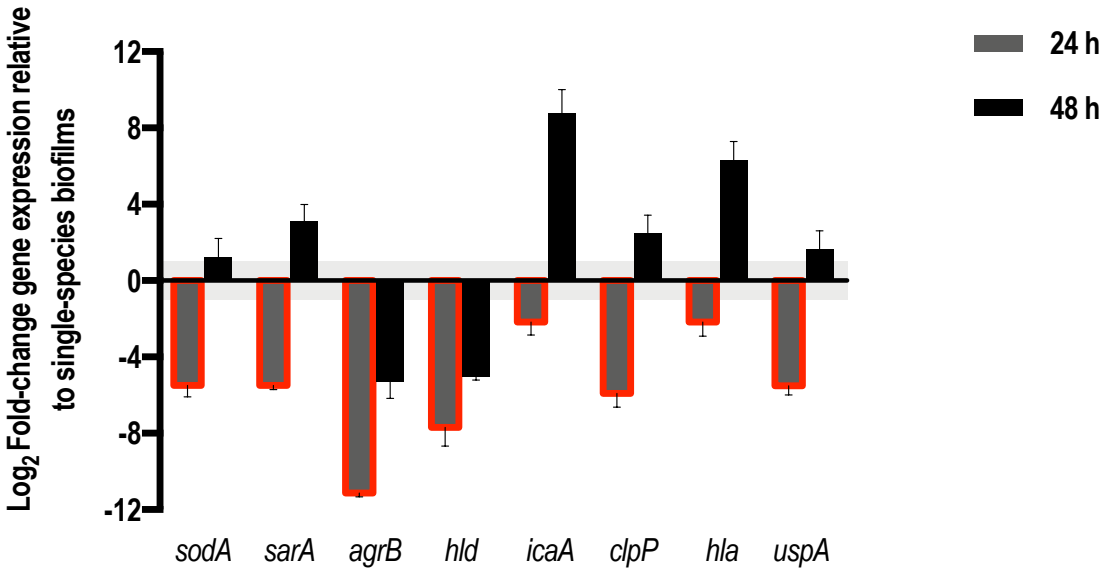

Gene	Function
sodA	Stress Response
sarA	Virulence Regulator (Quorum sensing)
agrB	Virulence Regulator (Quorum sensing)
hld	Virulence Regulator (Quorum sensing)
icaA	Biofilm formation (PNAG production)
hla	Alfa-hemolysin
uspA	Stress Response

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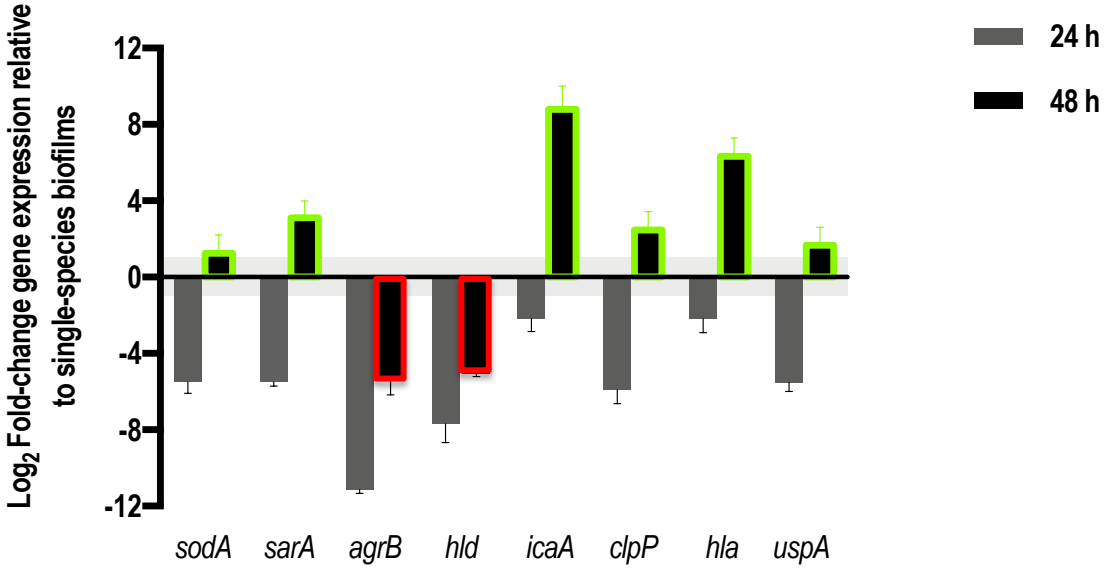

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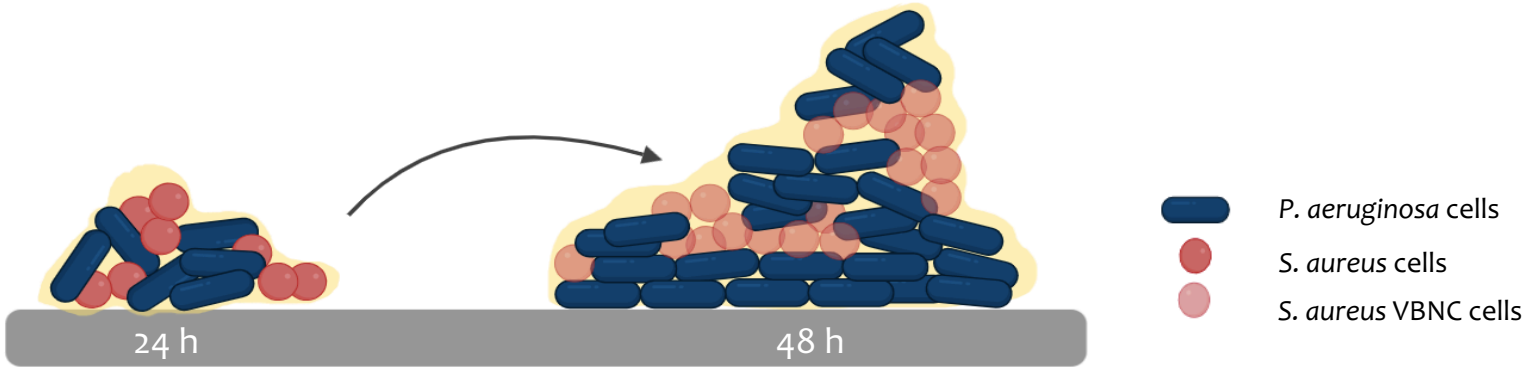
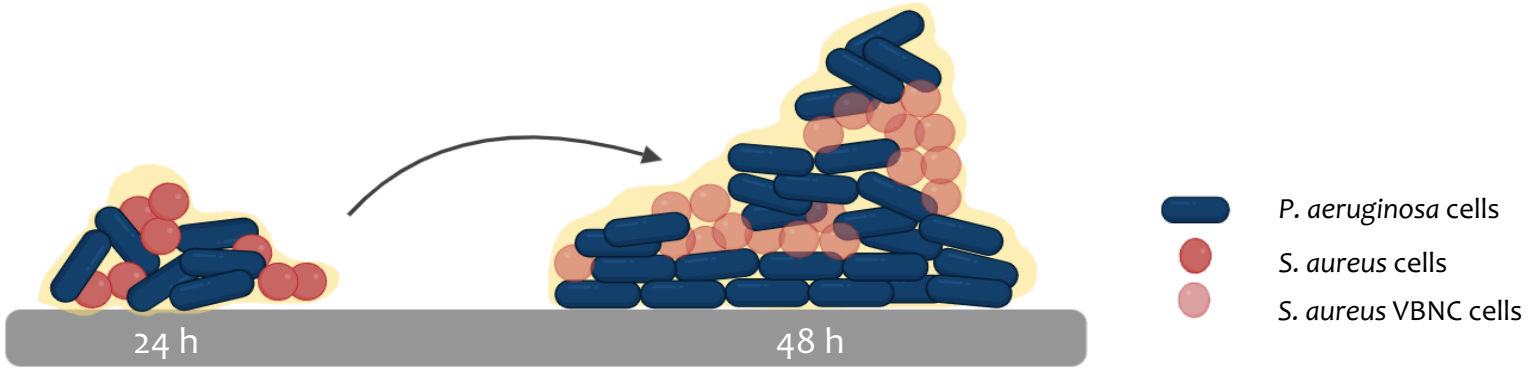
Conclusions

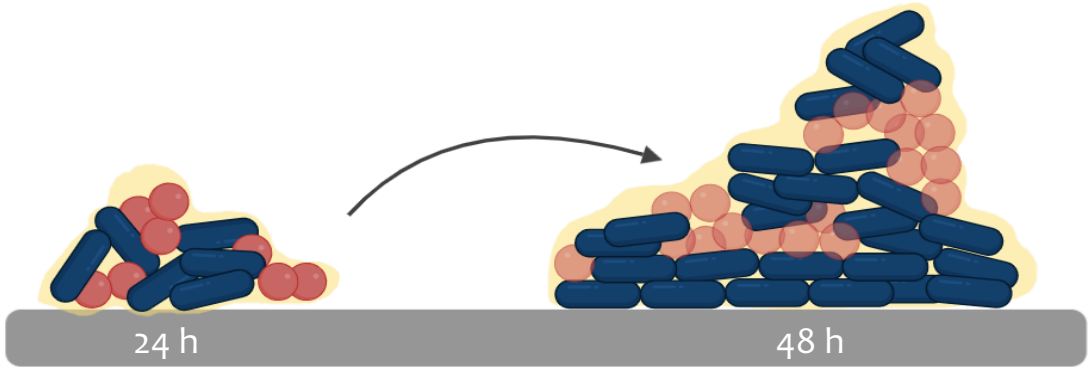


Conclusions



- ✓ Time-dependent interaction between *P. aeruginosa*-*S. aureus* in dual-species biofilms.
- ✓ The dual-species consortia dominated by *P. aeruginosa*
- ✓ The presence of *S. aureus* in high numbers in dual-species biofilms with *P. aeruginosa* in a VBNC state.

Conclusions



P. aeruginosa cells

S. aureus cells

S. aureus VBNC cells

Up-regulation
Virulence-related genes

P. aeruginosa

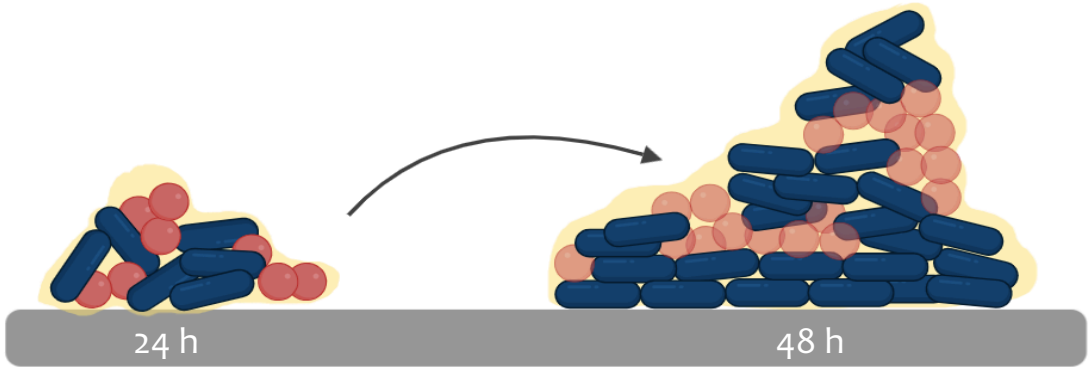
S. aureus

Down-regulation
Virulence-related genes

S. aureus

P. aeruginosa

Conclusions



P. aeruginosa cells
S. aureus cells
S. aureus VBNC cells

Up-regulation Virulence-related genes	↑ *P. aeruginosa*	↑ *S. aureus*
Down-regulation Virulence-related genes	↓ *S. aureus*	↓ *P. aeruginosa*

Overall, our results underline the importance of select appropriate methodologies to elucidate the microbial interactions occurring within the dual-species biofilm consortia.

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Supervisors : **Maria Olivia Pereira** (CEB, University of Minho, Portugal)

Nuno Cerca (CEB, University of Minho, Portugal)

MOP Team

NC Team

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UNIÃO EUROPEIA
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Thank you!

Andreia Magalhães

amagalhaes@ceb.uminho.pt