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Optimized *Pseudomonas aeruginosa*-*Staphylococcus aureus* mixed biofilm platform for dispersal agents screening

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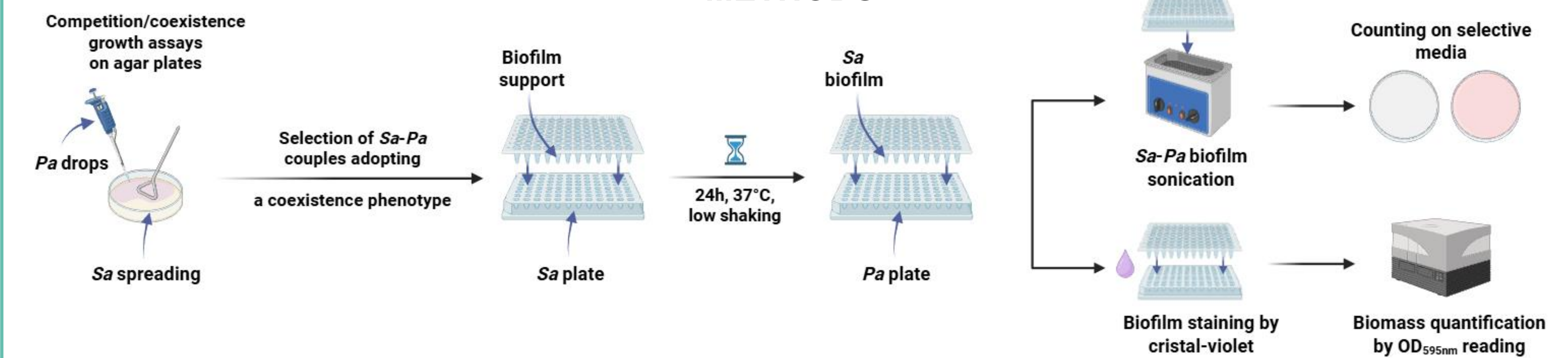
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INTRODUCTION AND OBJECTIVES

The use of exogenously added cues to promote biofilm dispersion has emerged as a promising strategy, as dispersed bacteria revert to the planktonic state and lose the biofilm-associated tolerance to antimicrobials^[1,2]. *Pseudomonas aeruginosa* (*Pa*) and *Staphylococcus aureus* (*Sa*) are opportunistic pathogens frequently co-isolated from chronic biofilm-associated infections. Their coexistence within biofilms enhances adaptability and resistance to a broad range of antimicrobial agents and host immune responses, making infections extremely difficult to eradicate and leading to poor clinical outcomes. Moreover, co-culturing these two species *in vitro* remains technically challenging, hindering their study. This work aims to establish and optimize a *Pa*-*Sa* *in vitro* dual-species biofilm model to serve as a screening platform for biofilm-dispersing agents.

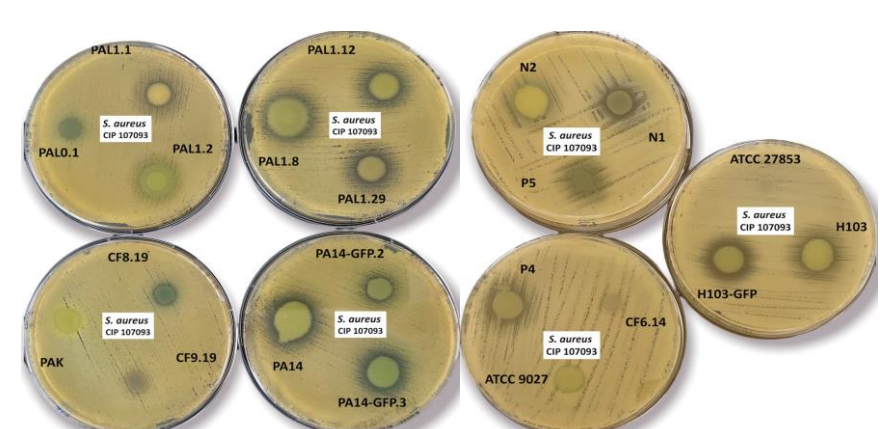
METHODS



RESULTS

1 Interaction tests between a panel of *Sa* and *Pa* strains

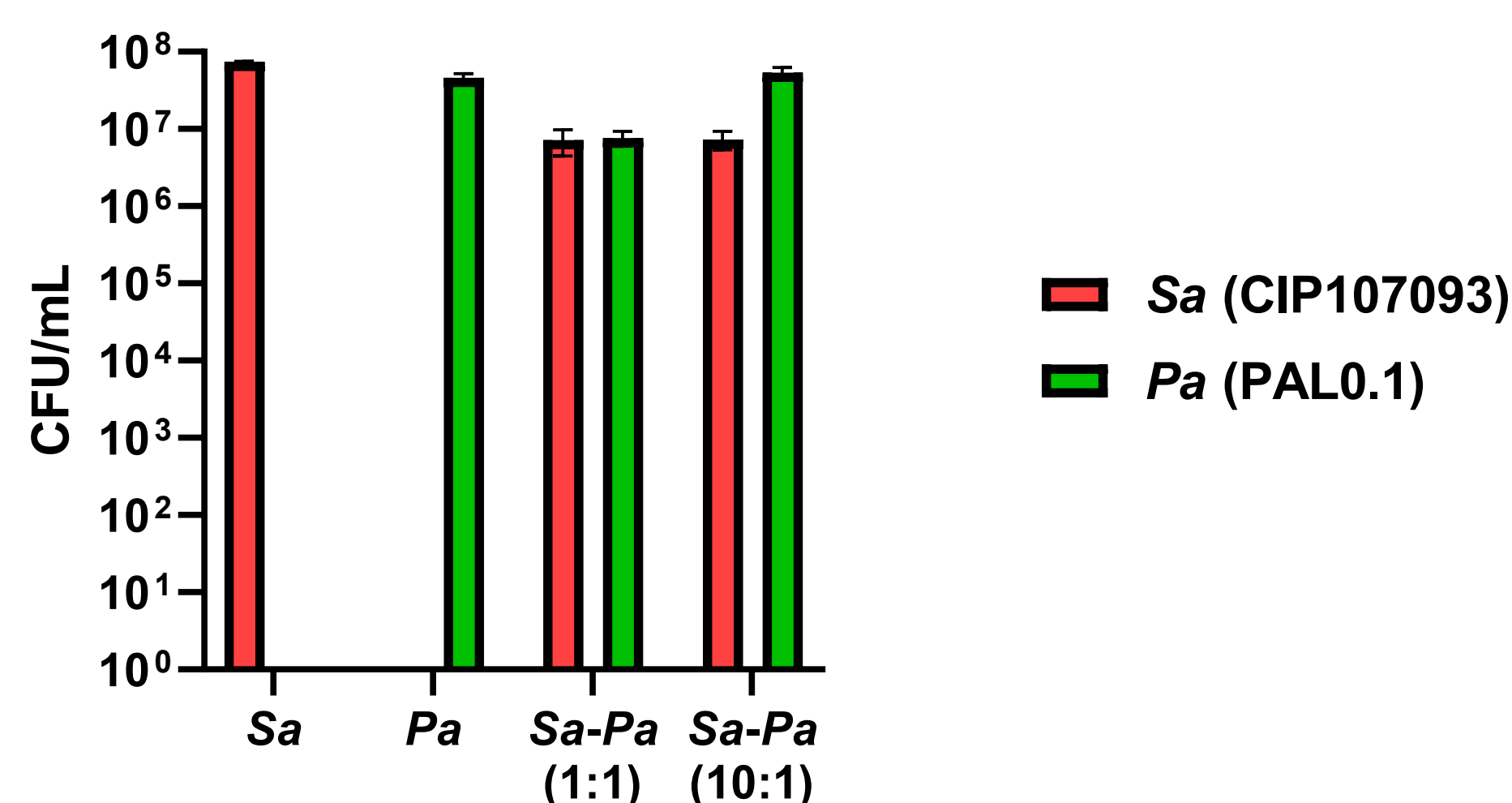
Pa-*Sa* couples of strains adopting a coexistent phenotype among a collection of reference and clinical isolates were selected using a competition/coexistence agar plate interaction assay.



The strain couple *Sa*(CIP107093)-*Pa*(PAL0.1) was selected for the development of the *Sa*-*Pa* dual-biofilm model.

3 Validation of a *Sa*-*Pa* dual-species biofilm model

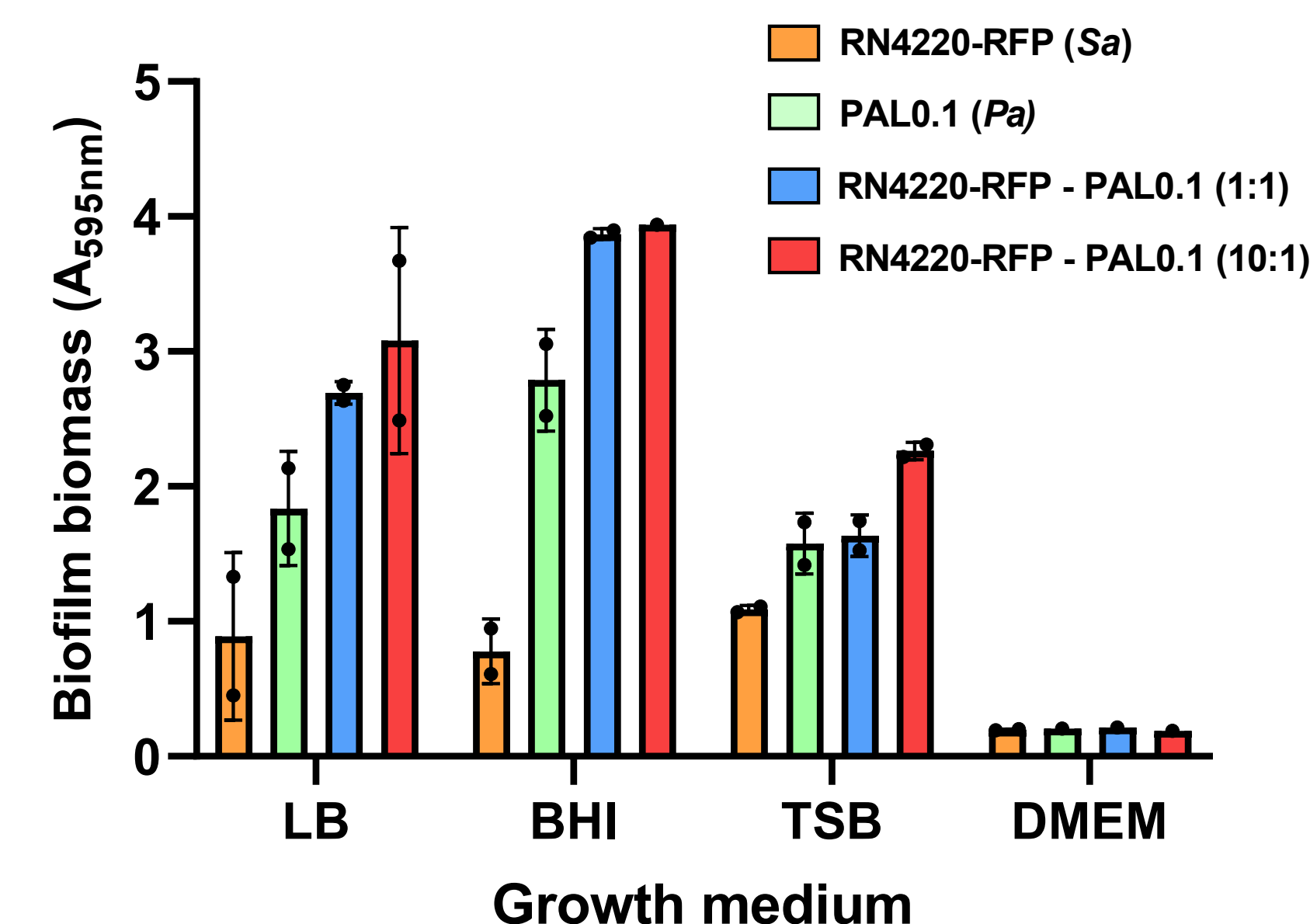
Bacterial enumeration of *Sa* and *Pa* in mono and dual-species biofilms. Biofilm were sequentially grown during 48h (*Sa* t=0h, *Pa* t=24h). Bacteria were inoculated at OD_{600nm}=0.1 or 0.01 to set the inoculum ratios. Biofilms were grown on polystyrene pegs and were disrupted by sonication. *Sa* and *Pa* were counted on mannitol salt agar and cetrimide agar media, respectively.



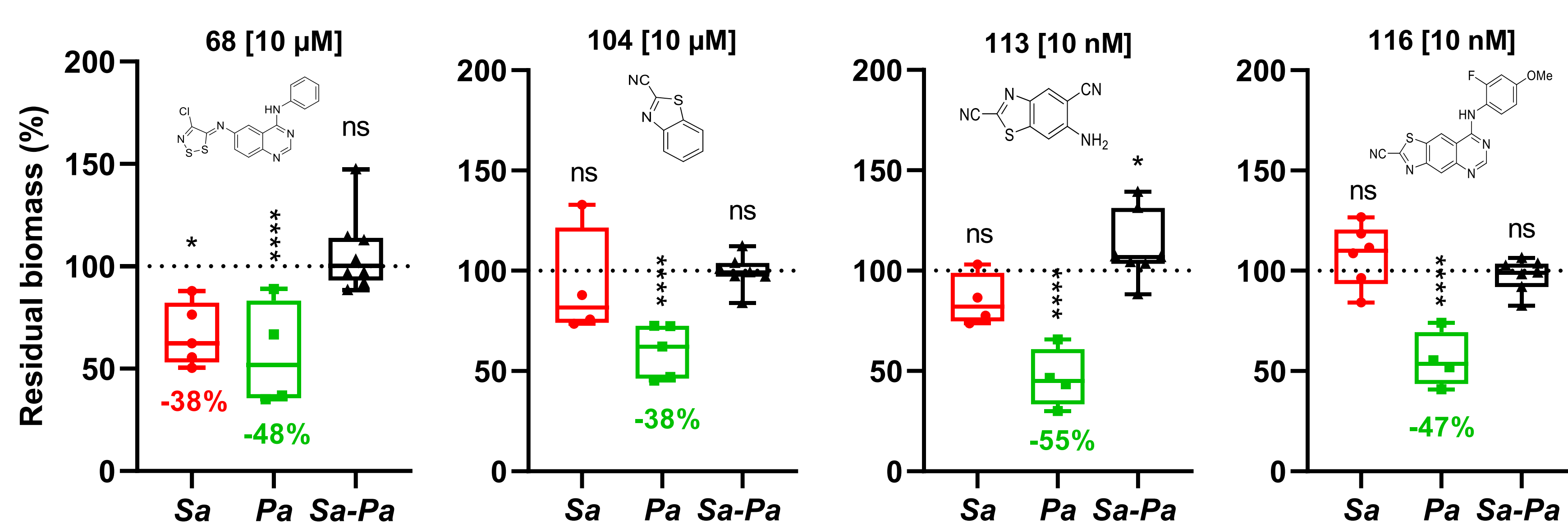
The optimised *Sa*(CIP107093)-*Pa*(PAL0.1) dual-species biofilm model was used as a platform screening of biofilm dispersion activity.

2 Optimisation of co-culture growth of *Sa* and *Pa* mono and dual-species biofilms growth

Mono and dual-species biofilms of *Sa* (RN4220-RFP)-*Pa* (PAL0.1) were grown at ratios of 1:1 or 10:1 in LB, BHI, TSB or DMEM at 37°C during 24h on static conditions. **BHI medium showed to be optimal for *Sa* biofilms growth. Shaking helps the non-motile *Sa* forming biofilm on peg support.**



4 Dispersal of *Sa* and *Pa* mono-species and dual-species biofilms



Mono and dual-species biofilms of *Sa*(CIP107093) and *Pa*(PAL0.1) were grown following the conditions of the model previously described. Biofilms were treated with *N*-imino-1,2,3-dithiazoles derivatives for 2h at 37°C. These compounds were already shown to induce dispersion on *Pa* PA14 biofilm^[3]. *, p<0.05 ; ****, p<0.001 ; ns, not significant.

All compounds induced significant *Pa* biofilm dispersion, but only 68 induced *Sa* biofilm dispersion.

No dispersion activity was observed on *Sa*-*Pa* biofilm.

CONCLUSION

This work allowed the development of an *in vitro* *Sa*(CIP107093)-*Pa*(PAL0.1) dual-biofilm model that we propose to use as a high throughput screening platform of biofilm dispersion activity. Further work is needed to improve this current model through its validation using clinical co-isolates of *Sa* and *Pa* and its establishment on *ex vivo* and *in vivo* experimental models that accurately replicate the physiologically relevant conditions of clinical biofilms, that would enable a more precise investigation of the dual-species biofilm dispersion.

References:

- [1] Tareau et al. (2025). *Biofilm*. 27;9:100293. doi: 10.1016/j.biofilm.2025.100293
 [2] Rumbaugh & Sauer (2020). *Nat. Rev. Microbiol.* 18(10):571-586. doi: 10.1038/s41579-020-0385-0.
 [3] Gonzalez et al. (2025). *Pharmaceuticals*. 18(11), 1733. doi: 10.3390/ph18111733

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